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HYDROGEN ION CONCENTRATION AND THE COMPOSITION OF NUTRIENT SOLUTIONS IN RELATION TO THE GROWTH OF SEED PLANTS

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INTRODUCTORY

By the use of the terms mineral nutrition or salt requirements of plants there is connoted a group of physiological processes and environmental conditions in which the mineral salts play an important rôle. Without entering into an elaborate discussion of these terms it may at least be pointed out that perhaps neither one is satisfactorily comprehensive. The last-mentioned is vague, and the other inadequate, due to the fact that when plants are grown in a so-called nutrient solution, as is well known, it is not merely the nutrient rôle the effects of which are followed. The concentration of the solute molecules and component ions of the culture solution directly affect the turgor, or osmotic surplus; the proportions of the ions—particularly of the cations—influence the permeability relations, which, operative through the protoplasts, seem to be the fundamental consideration in certain "antagonism" phenomena; the composition of the salts employed determines the acid-alkali equilibrium, that is, the hydrogen ion concentration, the influence of which is apparently most complex; and these and other possibilities affect ultimately growth, which, in part, of course, involves the incorporation into the living framework of the ions of the component salts.

For several years[1] the writer has given attention, as occasion permitted, to the reaction of the medium as affecting growth in soil solutions, also its effect upon toxic action; but it is only within the past two years that opportunity has been taken from time to time to examine this factor critically and so far as practicable in respect to physiologically balanced nutrient solutions.

The illuminating contributions of Clark and Lubs ('17) on the colorimetric determination of the hydrogen ion concentration of culture media for microörganisms, and of other biological fluids, effected so great an improvement in the simpler technique involving active acidity and alkalinity that there is now available a convenient, rapid, and sufficiently accurate method of investigating the hydrogen ion relations in connection with the salt requirements of higher plants. On the other hand, the extensive contributions of Schreiner and Skinner ('10, '10a, '11, '12), Tottingham ('14), Shive ('15), McCall ('16), and many others, on salt requirements and the constitution of the mineral nutrient solution have made it possible to select, within certain limits, well-balanced solutions as points of departure. In the work here reported, therefore, the writer has not concerned himself to any great extent with an investigation of the effects of variations in the proportions of the different salts involved in the nutrient solution. In this last-mentioned direction the recent literature represents a rapid advance both in technique and in result, and while, as will be pointed out later, the problem may not be finally solved, it has certainly been placed on a rational basis.

The triangle-diagram scheme was first rendered of biological significance by Schreiner and Skinner, referred to above, and it was effectively employed by them in studies of the relations of plants to certain toxic agents which might exist in the soil and in studies of the ameliorating action of the nutrient ions, K, NO_3, and PO_4, upon such deleterious compounds. They likewise investigated by this means the growth of wheat as affected by different ratios of phosphate, nitrate, and potassium. The same general diagram scheme has been perfected and advan-

[1] During the progress of these investigations the writer has had much assistance in the details of the culture work from various members of the graduate laboratory, to all of whom he is indebted, especially to Mrs. Emily Schroeder, Research Assistant, 1918–19, and Mr. R. W. Webb, Research Assistant, 1919–20.

tageously applied by Tottingham in his elaborate account of nutrient solutions, and by Shive, McCall, and others in intensive analyses of physiological balance. The effect of variation in H-ion concentration may be approached finally in the same way. In this rather general survey of certain aspects of the subject, however, it has seemed that the factors are for the most part too complicated for most economical treatment by this method alone.

Technique and Materials

In the earlier work I selected for comparative studies on the influence of variation in H-ion concentration two solutions only: namely, a slight modification of Shive's R_5C_2, considered his best solution of optimal concentration, and a much modified Crone solution developed in this laboratory. Later there was added the R_6C_1 solution of Livingston and Tottingham, and ultimately some other combinations which seemed worthy of consideration.

The partial volume-molecular proportions of the particular Shive solution employed are as follows: KH_2PO_4, 0.0180; $Ca(NO_3)_2$, 0.0052; and $MgSO_4$, 0.0150. Ferric phosphate in small amount is added, the partial concentration being 0.0044 gm. per liter of solution. This insoluble salt is used at such a low concentration that its presence as a precipitate is scarcely perceptible. I have employed the same salts in the same proportions, except that "soluble ferric phosphate" has been substituted for the insoluble iron salt in all cases not otherwise indicated. This substance is described as consisting of scales of ferric phosphate with sodium citrate, possibly as a single salt. The exact molecular composition of this iron-furnishing substance is unknown. From the description in the National Standard Dispensatory (1905) it will be seen that it is commonly made by the addition of 50 gms. ferric citrate and 55 gms. sodium phosphate, unefloresced, to 100 cc. distilled water. It is barely possible that four salts are present, namely, sodium and ferric phosphate and sodium and ferric citrate. This salt combination possesses the advantage of solubility to a high degree, yielding what appears at first to be a true solution but seems in reality a colloidal solution of high dispersity. Used in such extreme dilution as

indicated in the Shive solution, there is no noticeable precipitation, even on standing. There is every reason to believe that this substance is an excellent source of iron. The Shive solution prepared in this way is designated solution A, and it is best to think of it as solution A, because, as developed later, Shive does not report the P_H for his solutions, and this three-salt solution may possess very different values in plant growth, depending upon the grade or reaction of the monobasic phosphate employed.

The original Crone solution is made up as follows: water, 1000 cc.; KNO_3, 1 gm.; $CaSO_4$, .5 gm.; $MgSO_4$, .5 gm.; $Ca_3(PO_4)_2$, .25 gm.; and $Fe_3(PO_4)_2$ .25 gm. In our solution we have halved the concentration of the first two salts, omitted the tricalcium phosphate, and substituted for the ferrous phosphate the "soluble iron phosphate" above mentioned. In fact, the solution as I have used it may no longer be called the Crone solution, but it was of interest in this work at the time, not because it was expected to compare favorably with solution A above, but rather because it differed widely in the combinations of the components and had afforded in my work very good, healthy growth. The partial volume-molecular proportions are as follows: KNO_3, 0.00495; $CaSO_4$, 0.000726; $MgSO_4$, 0.000526; and "soluble iron phosphate," 0.125 gm. per 1000 cc. It will be noted that this is in reality a four-salt solution differing also notably from the earlier solutions of Sachs, Knop, Pfeffer, and Meyer. Inasmuch as the iron salt supplies also the phosphate, this B solution contains a higher concentration of Fe than the usual culture solution. There is more or less precipitation of a light or slowly settling iron salt, when the combined solution is prepared. However, by adding the soluble ferric phosphate last and at the greatest dilution possible under the conditions a light precipitate only is formed, and this develops slowly in the form of suspension films. It introduces no difficulties into the preparation of the solution, though perhaps renders its composition somewhat less definite. The films are so light that it is not difficult to remove uniform samples from the stock flasks. The osmotic value of this solution has not been determined, but it is obviously much less than that of the Shive solution, the latter being about 1.75 atmospheres.

The solution of Livingston and Tottingham, here designated solution C, possesses the following partial volume-molecular concentrations: KNO_3, 0.0216; $Ca(H_2PO_4)_2$, 0.0026; and $MgSO_4$, 0.0150. Iron is supplied as in the case of solution A, the modified Shive solution. It is assumed that this solution has approximately the osmotic value of solution A above described.

The methods of experimentation employed were in large measure those which I have described elsewhere ('11). "Well-seasoned" tumblers holding somewhat more than 250 cc. were used as culture vessels in all cases, the process of seasoning new tumblers consisting of filling them with a weak acid-dichromate cleaning solution and then steaming in the autoclave for one hour at 15 pounds pressure. Subsequently the tumblers were thoroughly washed and rinsed with distilled water. The distilled water used here and in preparing the solutions was in some series from a Stokes still and in other series double distilled from glass. After transferring, with pipette or burette, the required amounts of each constituent stock solution to the tumbler, the volume was made up to 240 cc. In those cases in which Canada field peas were used the tumblers were covered with heavy paraffined paper made fast by rubber bands. Small holes were punched in the paper and the radicles of the seedlings inserted. Additional support for the growing seedlings was afforded by means of wire supports. In the case of both wheat and corn the seedlings were inserted into notches or holes in a paraffined cork, the latter just fitting the mouth of the tumbler. All cultures contained ten plants, and duplicate tumblers were arranged in every case not otherwise indicated.

In most of the experiments here reported Merck's blue label chemicals have been employed, but under the conditions existing at the time it was not possible to be entirely uniform in this regard, and other standard reagents were used where necessary. No recrystallization or other purification method was applied to any salt employed in the culture solutions. Stock solutions of convenient concentration were prepared, and so far as practicable every factor and procedure was made uniform, or comparable, throughout. It soon became evident that the content of free phosphoric acid in the dihydrogen potassium phosphate

was not constant in the different makes and bottles of the reagent necessarily used, and a closer examination of this point as the work progressed indicated clearly that we were not securing the uniformity of P_H in the solution which we felt that we might reasonably expect. It was not anticipated that the standard as indicated by Sörensen ('09–'10) and others would be attained unless the reagent were guaranteed free from phosphoric or other acids. The variation in P_H is often too great to make it at all certain what is meant when this salt is designated merely as acid potassium phosphate. One manufacturer who was appealed to on account of the variability referred to, assured the writer that it was not possible to furnish the salt free of phosphoric acid under the existing conditions.

In this work the hydrogen ion concentration was invariably determined by the method of Clark and Lubs ('17), employing both their standard solutions and their indicators. The standard solutions in this connection, however, were prepared with salts recrystallized two or three times, and the greatest care was given to every detail of the method. They were not controlled by the electrode method but by close comparison with the established effective ranges of the different indicators, especially, also, indicators with overlapping ranges. When making comparisons there was arranged for each indicator a set of seasoned serological test-tubes each containing 5 cc. of any standard solution within the range of the particular indicator. The various sets were arranged on a rack with white paper background. From 3 to 5 drops of the indicator solutions were used. Inasmuch as many of the culture solutions employed were without color, sufficiently accurate determinations were readily made with the unaided eye. In the case of solution B, however, and likewise in certain cases referred to later, where a few algae appeared in the solutions, recourse was had to the method employing the colorimeter (Duggar and Dodge, '19) and later more especially to the micro-colorimeter (Duggar, '19).

The seedlings used in various phases of the work were wheat (*Triticum vulgare*), corn (*Zea Mays*), and field peas (*Pisum arvense*). In all the work reported in tables I–VI, also XI–XIX, the wheat was of the variety Fultz, and that reported in tables VII–X was with a new variety, the Pacific Coast Blue Stem,

supplied from the Plant Introduction Garden of the Bureau of Plant Industry at Chico, California. The latter had been found by Dr. H. S. Reed to be particularly good for solution culture work, and my experience is entirely confirmatory. The other seed were from selected but unnamed field varieties.

The seed were immersed over night in running water, and then the peas and corn placed for germination on paraffined wire netting over pans of well-washed, moist sphagnum. They were covered with moistened paper toweling, over which was inverted other pans, though ample ventilation was provided. The wheat was treated in much the same way except that it was germinated over water frequently changed. As soon as the plumules emerged light was admitted. After properly placing the seedlings, ten to each tumbler, they were left in diffuse light, in the room in which set up, for 12 to 24 hours in order to become better adjusted to the conditions before being installed in the greenhouse.

In some of the earlier experiments a complete series was arranged under a single set of conditions, and in such cases the tumblers were placed upon a rotating table. In much of the later work, however, more than one set of conditions was involved, so that the use of rotating tables was not practicable, and under the circumstances special care was taken with properly placing and spacing the tumblers on lattice tables, likewise shifting the order of the cultures so as to be wholly comparable.

In all cases as the plants were harvested, the remains of the seed, or cotyledons, were cut away. This was found necessary inasmuch as otherwise a considerable error would be introduced in the weights of those cultures in which relatively little growth occurred; for it was in such plants that the seed were incompletely exhausted. On removal from solutions containing precipitates the roots were thoroughly washed, and in all cases quickly and uniformly dried of surface water on absorbent gauze.

The criterion of growth on which stress is laid in this paper was total green weight. Other data included green weight of tops, average length of shoot (or leaf), dry weight, and general appearance—including root characteristics. Total green weight is, for the present purpose, entirely satisfactory and has the advantage in these relatively short-interval cultures of expressing

as truly as may be the growth and health of the plants. Dried leaves and withered tips count for little. Average length of leafy shoot is included in some of the tables, but this affords merely an index of stockiness or attenuation of the plants.

The purpose in this work was to get results not only in respect to growth relations with variations in the H-ion concentration, but also, especially in the later work, to secure data in respect to the extent of change in the P_H of the medium in which the plants had grown. The time interval which the plants were permitted to remain in the cultures between changes of solution was generally 6 to 7 days, but in certain cases referred to later this interval was diminished or increased for special reasons indicated in connection with the tabulated data. During any interval, however, distilled water was added as required. In general, it seemed to the writer that the practical value of a particular mineral nutrient solution should rest in part upon its capacity to furnish favorable growth conditions for a period not too limited, that is, the solution should possess among other favorable characteristics, if possible, the quality of resisting unfavorable change over a period of one week or even longer.

Had the nutrient solutions used been merely solutions A and C, in which phosphate is supplied as the acid salts, it would have been simple to change the H-ion concentration towards neutrality by the use of various proportions of a dibasic salt. However, it was at the same time desired to increase the H-ion concentration, that is, to diminish the P_H exponent in order to determine approximately the favorable P_H limits. Moreover, changes in solution B could not be readily effected in the manner indicated without material changes in composition. It was therefore determined to use, in general, 0.1 n NaOH and approximately 0.1 molar H_3PO_4 in shifting the H-ion concentration. Actually, the concentrations employed were 0.1 n NaOH and 0.092 molar H_3PO_4. The stock solutions were so arranged as to concentration that the amounts of alkali or of acid introduced might first be diluted considerably with distilled water. In this way there was less danger of precipitating out the calcium salt, as insoluble calcium phosphate, for example, in solution A, before the critical P_H was reached, also other possibilities of precipitation in the case of solution B.

The problem was then to shift the hydrogen ion concentration of the solutions used without disturbing any more than necessary the composition of the medium. From preliminary tests of solutions A and B it was inferred that the chief interest might attach to the addition of alkali to solution A and of acid to solution B. The P_H exponent of the former is low, but in the latter it more nearly approaches neutrality. It is seen that the active acidity of the Shive solution is due to dihydrogen potassium phosphate, and the relatively slight variation in the proportions of the other practically neutral salts may be assumed not to change materially the hydrogen ion concentration. In this sense, therefore, the 108 solutions tested by Shive are probably nearly identical and determine, in respect to growth, the values of the proportions of the nutrient ions present in the solutions only in relation to this one value of P_H. This value might, of course, be assumed to be most favorable under all environmental conditions, but it is equally possible that it is not. This is a factor which should be given most careful consideration in all mineral nutrient, or salt-balance, studies as well as in studies upon toxic action.

As a convenient index to the content of the various cultures a simple scheme of notation has been devised, which, with very slight call upon the memory, enables one to see at a glance the constitution of the culture medium. This involves a system of letters and numerals as explained below. The initial letter of a culture refers to the general constitution of the nutrient solution, and as stated above there are three such solutions. The modified Shive solution employed is designated A; the solution remotely based on the Crone formula is solution B; and the Livingston-Tottingham solution, C. The next letter is invariably a small letter and denotes the plant employed; thus w designates wheat; p, Canada field peas; and c, corn; while x is employed where more than a single kind of plant is designated, or where no particular plant is specified. A culture index such as ApO, BpO, or CpO, etc., refers to the use of peas with the solutions mentioned without the addition of acid, alkali, or other constituent. When acid or alkali is added an inclined line represents, in this case, neutrality, and when figures are given above the line mentioned they represent cc. of 0.092 molar phosphoric

acid; and when given below they represent cc. of 0.1 n sodium hydroxide. Thus Ap/10 indicates the addition of 10 cc. of 0.1 n NaOH to the A solution with peas. For the purpose of this paper we have considered molar phosphoric acid to be the sum of the values derived by titration against normal alkali, using as indicators both phenolphthalein and methyl orange.

I have attempted to control or counterbalance, in a measure, the addition of phosphorus when added as phosphoric acid by the introduction in one or two cultures of an amount of phosphorus in the form of the secondary salt equivalent to the acid added. At the same time, however, the addition of this salt necessarily shifts slightly the hydrogen ion concentration toward neutrality. The addition of sodium in the form of the alkali was in a measure controlled by adding as sodium sulphate an amount of this cation which is equal to that supplied in 10 cc. of alkali. As the work has progressed some other variations have been employed, notably the addition of solid calcium carbonate (1 gm. per culture, 240 cc.) to solution B, likewise of solid aluminium hydroxide, and of kaolin, the addition of phosphate in part as the primary salt and in part as the secondary salt, also variations in the form and amount of phosphate employed, and significant changes in the proportions of solution B. Modifications of the culture indices denoting such changes will be explained as these are introduced.

EXPERIMENTAL DATA

There are given in tables I, II, and III the results of the first tests conducted with wheat, corn, and peas respectively, using solutions A and B. In consulting these tables it is to be remembered that the "culture indices" are intended to afford briefly all necessary facts concerning the constitution of the solution, and an explanation of these has been made on p. 9, so far as the unmodified solutions and those containing additions of acid and alkali are concerned. In the tables referred to above, K/10 and K/20 represent respectively additions of K_2HPO_4 to balance the amounts of phosphoric acid added in cultures with similar numerals; and Na indicates the addition of sodium sulphate to equal the quantity of gram atoms of Na in 10 cc. of n/10 NaOH.

The wheat cultures were grown 17 days, the corn 21, and the

peas 18. The solutions were renewed and water loss was supplied as previously noted. The determination of the hydrogen ion concentration "after" growth in the case of corn furnishes an index of the change in the solution after the plants had grown in it for 10 to 12 days, representing the final interval. In this particular case the intervals between changes of solution were made rather long, with the idea of emphasizing conditions. The experiments were begun early in November under favorable growing conditions. The daily evaporation rate from a standardized spherical evaporimeter averaged 11 cc. per day.

TABLE I
(Series 1, Wheat)

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH; SOLUTIONS A AND B

No.	Culture indices	Total gr. wt. (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	Initial P_H of sol.
1	AwO	4.92	0.4077	24.32	4.0
2	Aw /5	4.62	0.3916	23.89	5.6
3	Aw /10	5.20	0.4135	23.69	5.9
4	Aw /15	3.94	0.3550	21.24	6.1
5	AwNa	4.72	0.3792	25.93	4.0
6	BwO	4.52	0.3545	24.19	7.0
7	Bw /5	4.94	0.3498	23.36	8.4
8	Bw /10	5.83	0.4090	22.27	9.0(+)
9	Bw 5/	2.53	0.2522	16.48	4.6
10	Bw 10/	0.69	0.1176	8.26	3.2
11	Bw 20/	0.67	0.1134	6.56	3.0(−)
12	$BwK_2/10$	6.38	0.4390	26.34	6.2
13	$BwK_2/20$	5.80	0.3996	25.94	6.2

From series I, table I, also fig. 1, it is seen that with wheat there is indication that the addition of a certain amount of alkali, shifting slightly the H-ion concentration, is beneficial, though Aw/5 is irregular. Wholly unexpected is the extent of the growth in certain of the B cultures. Introduced empirically, this solution has not only yielded well, but in their vigorous, green appearance these cultures rank highest. The addition of alkali to solution B is beneficial, possibly from the addition of the sodium ion or from the slight increase in the concentration

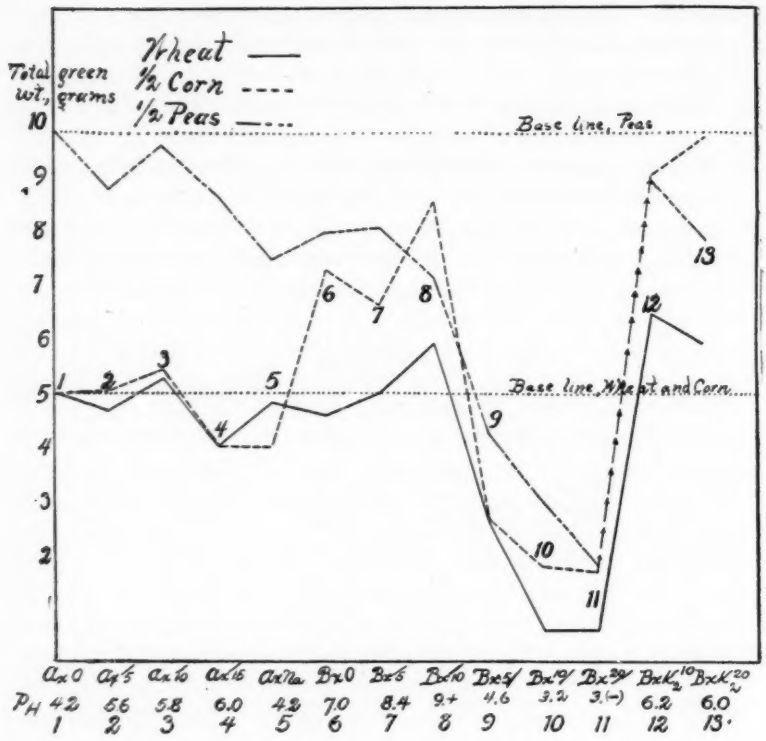

Fig. 1. Total green weight of wheat, corn (½), and Canada field peas (½), in solutions A and B, with variations—chiefly in H-ion concentration.

of the solution; and even more favorable is the addition of small amounts of secondary phosphate. With corn, table II, the shift towards alkalinity in the A solution affords slightly increased growth, but it is an important fact that there is a rapid falling off in the neighborhood of P_H 6. This is approximately the H-ion concentration at which precipitation begins. With this crop, the B solution alone, solution B plus small amounts of alkali, and solution B with the addition of dibasic phosphate yield strikingly heavier than solution A. On the other hand, peas, under the conditions of these experiments, made maximum growth in the relatively acid solution A.

TABLE II
(Series 1, Corn)

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH: SOLUTIONS A AND B

No.	Culture indices	Total gr. wt. (gms.)	Gr. wt. of roots (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H	
						Initial	After gr.
1	AcO	9.84	2.91	0.5452	22.02	4.0	5.4
2	Ac /5	9.94	2.74	0.6139	22.64	5.6	5.6
3	Ac /10	10.71	2.43	0.7642	22.97	5.9	5.9
4	Ac /15	7.90	2.25		20.36	6.1	6.0
5	AcNa	7.92	2.56	0.4652	14.49	4.0	5.2
6	BcO	14.35	4.22	1.0198	25.79	7.0	5.0
7	Bc /5	13.12	2.70	0.8102	26.28	8.4	8.0
8	Bc /10	16.90	3.95	1.0271	29.24	9.0(+)	8.4
9	Bc 5/	5.23	1.17	0.4606	14.67	4.6	4.0
10	Bc 10/	3.43	0.70	0.5387	10.54	3.2	4.0
11	Bc 20/	3.32	0.41	0.5617	8.42	3.0(−)	4.0
12	$BcK_2/10$	17.75	3.94	1.0170	31.84	6.2	7.2
13	$BcK_2/20$	19.22	4.51	1.1881	30.09	6.2	6.6

TABLE III
(Series 1, Peas)

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH: SOLUTIONS A AND B

No.	Culture indices	Total gr. wt. (gms.)	Gr. wt. of roots (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	Initial P_H of sol.
1	ApO	19.47	6.44	1.1014	27.84	4.0
2	Ap /5	17.37	5.31	0.9368	27.92	5.6
3	Ap /10	17.71	5.51	1.0155	29.42	5.9
4	Ap /15	17.10	5.42	1.0140	26.76	6.1
5	ApNa	14.77	5.57	0.8786	25.60	4.0
6	BpO	15.72	5.76	0.9200	25.10	7.0
7	Bp /5	15.86	5.10	0.8559	27.40	8.4
8	Bp /10	14.10	4.56	0.7822	25.59	9.0(+)
9	Bp 5/	8.27	2.70	0.6558	15.83	4.6
10	Bp 10/	5.85	1.78	0.5184	12.98	3.2
11	Bp 20/	3.47	0.93	0.3770	7.59	3.0(−)
12	$BpK_2/10$	17.69	5.17	0.9770	29.19	6.2
13	$BpK_2/20$	15.30	4.79	0.8660	27.18	6.2

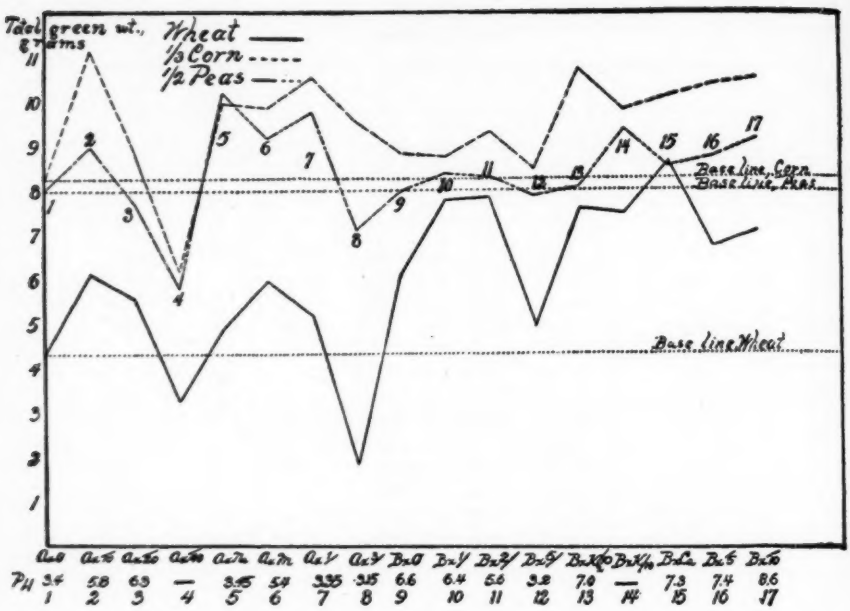
Total green wt., grams
Wheat
½ Corn
½ Peas
Base line, Corn
Base line, Peas
Base line, Wheat
A10 A9 A8 A7 A6 A5 A4 A3 A2 A1 B10 B9 B8 B7 B6 B5 B4 B3 B2 B1
PH 3.4 5.8 6.9 — 3.45 5.4 3.35 3.15 6.6 6.4 5.6 3.8 7.0 — 7.3 7.4 8.6
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Fig. 2. Total green weight of wheat, corn (½), and Canada field peas (½), in solutions A and B, with variations—chiefly in H-ion concentration. The base lines are drawn through cultures strongly acid, P_H 3.4.

A point of considerable interest, emphasized particularly by the results with peas, is that the range of most favorable growth with respect to hydrogen ion concentration differs materially with the constitution of the nutrient solution; thus the addition of acid to the B solution, although shifting the H-ion concentration towards that of the A solution, exhibits a corresponding rapid diminution of the growth quantities. From these data alone it is not possible to formulate an explanation of the fact last mentioned, but it is probably related in part to ionic conditions, in part to the composition and state of aggregation of the iron and calcium particles, or to other, indetermined factors.

It should be pointed out, that in examining the curve, fig. 1, and all subsequent curves, a horizontal, or base line, is drawn through the growth quantity representing generally the unmodified solution A, so that all cultures may be compared with this,

TABLE IV
(Series 2, Wheat)

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH

No.	Culture indices	Total gr. wt. (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	Initial P_H of sol.
1	AwO	4.39	.545	24.37	3.4
2	Aw /10	5.30	.611	24.75	5.8
3	Aw /20	4.56	.533	22.74	6.3
4	Aw /40	3.21	.431	19.80	*
5	AwNa	4.86	.556	25.01	3.45
6	AwM	5.93	.542	23.04	5.4
7	Aw 1/	5.21	.569	24.63	3.35
8	Aw 3/	1.79	.306	16.38	3.15
9	BwO	6.07	.503	21.86	6.6
10	Bw 1/	7.76	.625	23.96	6.4
11	Bw 2/	7.82	.657	24.40	5.6
12	Bw 5/	4.96	.500	21.81	3.2
13	$BwK_2/10$	7.60	.623	26.18	7.0
14	BwK /10	7.51	.602	25.45	
15	BwCa	8.67	.810	25.32	7.3
16	Bw /5	6.75	.570	23.24	7.4
17	Bw /10	7.08	.606	24.49	8.6

* Heavy precipitate.

and the number of points in the curves above or below the base lines indicates for each crop, under the conditions reported, the relative increase or decrease in green weight.

The change in the P_H of the solutions occurring as a result of contact with the roots was followed only in the case of corn. As a rule, in the more acid solutions the reaction is shifted somewhat towards neutrality, but irregularities occur in solution B, some of which may be related to changes not due to the interchange of ions between roots and solution.

The second series of experiments, the results of which are included in tables IV, V, and VI, also plotted in fig. 2, was carried through during late November and early December. The conditions were much the same in general as those prevailing during the earlier work. There was this difference, however, that while the first series was placed on latticed tables in greenhouses with proper spacing to provide for favorable and uniform condi-

TABLE V
(Series 2, Corn)

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH

No.	Culture indices	Total gr. wt. (gms.)	Gr. wt. of roots (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	Initial P_H of sol.
1	AcO	25.44	7.63	2.325	32.33	3.4
2	Ac /10	33.52	7.96	2.567	32.91	5.8
3	Ac /20	26.14	6.36	2.170	30.71	6.3
4	Ac /40	18.28	5.10	1.642	22.26	*
5	AcNa	29.94	7.89	2.001	32.49	3.45
6	AcM	29.48	8.10	2.139	27.57	5.4
7	Ac 1/	31.56	8.18	2.337	32.56	3.35
8	Ac 3/	28.60	6.06	1.888	32.06	3.15
9	BcO	26.46	8.60	2.515	33.71	6.6
10	Bc 1/	26.19	9.34	2.232	33.31	6.4
11	Bc 2/	27.86	10.29	2.151	33.03	5.6
12	Bc 5/	25.35	7.75	2.108	30.50	3.2
13	BcK_2/10	32.25	11.48	2.476	33.51	7.0
14	BcK /10	29.48	9.96	2.453	29.46	
15	BcCa	30.42	11.80	2.852	34.55	7.3
16	Bc /5	31.20	10.68	2.530	34.65	7.4
17	Bc /10	31.55	9.90	2.767	33.98	8.6

* Heavy precipitate.

tions, the second series was placed upon the rotating table and rotated throughout the period of culture. The rotating table employed was that previously described (Duggar and Bonns, '18) except that there was substituted for the special pot platforms a continuous platform constructed above the radiating arms and the secondary motion was, of course, eliminated. The wheat was grown 21 days, while the corn and peas were grown 24 days. One change of solution was made after about 12 days of growth. It is, therefore, a rather severe test of growth quantities when infrequent renewals of solutions are made. The water loss from transpiration was supplied about every second day.

In determining the hydrogen ion concentration of the solutions employed at the beginning of the experiment it was found that the active acidity of solution A was 3.4, consequently much greater than the theoretical. This was found to be due to the

TABLE VI
(Series 2, Peas)

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH

No.	Culture indices	Total gr. wt. (gms.)	Gr. wt. of roots (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H	
						Initial	After gr.
1	ApO	16.01	5.29	1.351	24.02	3.4	4.6
2	Ap /10	17.89	6.21	1.473	22.72	5.8	5.8
3	Ap /20	15.34	5.30	1.332	19.93	6.3	6.0
4	Ap /40	11.55	4.68	1.124	12.49	*	6.8
5	ApNa	20.30	7.20	1.603	26.03	3.45	4.4
6	ApM	18.28	6.15	1.418	24.19	5.4	6.4
7	Ap 1/	19.39	6.60	1.505	24.50	3.35	4.6
8	Ap 3/	14.17	5.22	1.262	21.13	3.15	4.2
9	BpO	15.91	5.70	1.326	19.59	6.6	6.2
10	Bp 1/	16.69	6.13	1.410	22.16	6.4	6.0
11	Bp 2/	16.58	5.82	1.391	22.48	5.6	6.0
12	Bp 5/	15.78	5.33	1.316	20.24	3.2	4.6
13	$BpK_2/10$	16.12	5.91	1.413	19.01	7.0	6.6
14	BpK /10	18.75	6.87	1.501	21.33		5.2
15	BpCa	17.15	6.18	1.393	23.44	7.3	8.0
16	Bp /5	17.49	6.32	1.389	19.21	7.4	7.4
17	Bp /10	18.38	6.29	1.413	21.65	8.6	8.0

* Heavy precipitate.

use of a new supply of monobasic potassium phosphate. This was a high-grade reagent, but was not guaranteed free of phosphoric acid, and no such guaranteed salt was then obtainable. The determinations indicate very clearly that attention must be paid to the determinations of the P_H value whenever such experiments on nutrition are conducted. This series of experiments must be examined and interpreted in the light of the P_H value referred to. Nevertheless, it should be pointed out here, though emphasized later, that the P_H exponent of the more acid solutions is rapidly increased with the growth of the crop. The increased acidity is doubtless due to an excess of acid in the preparation of the salt. This relatively high acidity affected, of course, to a degree the P_H of other solutions to which alkali was added.

In general, this series is an extension and continuation of the previous work, modified particularly by the addition of certain

cultures not included in the previous series. Attention may be drawn to the inclusion of cultures in which the hydrogen ion concentration of the A series was increased by slight additions of phosphoric acid, as in Ax 1/, Ax 3/, likewise in solution A the substitution for one-half the quantity of the monobasic salt by an equivalent of the dibasic potassium phosphate, this being designated AxM. In the B solution there were also introduced cultures to which relatively small amounts of phosphoric acid were added, Bx 1/, Bx 2/, and Bx 5/, also one culture in which was included 1 gm. of solid calcium carbonate, BxCa. A fresh quantity of the salt in the case last mentioned was introduced with each change of solution. A general examination of the total green weight quantities in the case of wheat indicates that under the conditions of this experiment the maximum growth quantities were obtained with the B solution. Small amounts of phosphoric acid or of either phosphate ($BxK_2/10$ and BxK/10) gave an increase in growth over the unmodified solution, and within the range of hydrogen ion concentration which prevailed in this culture solution relatively little influence was exerted by changes in P_H except in the case of one culture, Bw 5/, where the hydrogen ion concentration was increased to P_H 3.2. On the other hand, with the A solution it is clear that the addition of alkali to the unmodified solution is generally beneficial, at least at concentrations up to and including Aw /10, in which culture the P_H exponent is raised to 5.8. Further addition of alkali gives a falling off in the growth quantities. In the culture Aw /20 where the hydrogen ion concentration is P_H 6.3, precipitation occurred and a marked decline in growth is apparent, although the reaction of the medium is the same as that which in the B series promoted an amount of growth approaching the maximum. This is one of the many indications pointing clearly to the probability that the most favorable hydrogen ion concentration, or range of concentration, for a particular nutrient solution does not necessarily correspond to that which is most favorable with the solution of entirely different constitution. The growth quantity recorded for Aw 1/ is not strictly in line with the discussion above. The duplicates differed considerably. We have here, of course, two factors involved: (1) increase in acidity, and (2) slightly increased PO_4 concentration.

With respect to corn it will be seen that the maximum yield occurs in culture Ac /10, although maximum root growth occurs in several cultures. As between the different cultures in the A series the results are much the same as in the case of wheat except that the AcM culture with a combination of mono- and dibasic phosphate does not exhibit the benefit reported in the previous case. On the other hand, when one examines the data for dry weight quantities it will be found that maximum growth, as before, occurs in the B solution and in the culture to which solid calcium carbonate was added. Moreover, the next best growth is found in the addition of a slight amount of alkali to the B solution.

Throughout this work it will be noticed that the variation in total growth amounts between the various cultures of peas, differing mainly in P_H, is not so marked as that shown by the other two plants used in these experiments. Again, there were considerable differences between different plants in the same culture (more marked, however, in the case of wheat), and this indicates beyond any doubt that the variability of the seed is a factor which may affect to a slight extent the regularity of the results. In any event, the maximum growth with peas in this series occurred when a small amount of sodium sulphate was added. The amount of growth in the unmodified A solution is considerable in spite of the high acidity, yet there appears to be a slight advantage in the addition of a small quantity of alkali, although the latter is rendered doubtful by a comparison of cultures ApO, Ap 1/, and Ap 3/. In solution A Canada field peas are apparently only slightly affected by changes in hydrogen ion concentration up to the point of precipitation of the phosphate as the insoluble calcium salt. The curve, fig. 2, exhibits all the necessary data for growth comparison. Since these experiments were made it has been found that a frequent laboratory grade of acid potassium phosphate will give a P_H anywhere from 3.5 to 4.5, but more frequently less than 4.0.

Change in P_H after plants had grown in the solution was followed in the case of peas, with a result much like that of the first series. The more acid solutions are shifted towards neutrality, but solutions with exponents greater than 5.8 or 6.0 may vary scarcely at all, while the more alkaline solutions are generally shifted toward neutrality.

TABLE VII
(Series 3, Wheat)

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH; SOLUTIONS A, B, AND C; RENEWAL EVERY FOUR DAYS

No.	Culture indices	Total gr. wt. (gms.)	Total drywt. (gms.)	Greatest length (cm.)	P_H		
					Initial	End 2nd per.	End 4th per.
1	AwO	1.92	0.325	12.02	4.8	4.8	4.8
2	Aw 9/10 K	7.92	1.054	18.04	5.3	5.6	6.0
3	Aw 4/5 K	6.27	0.776	17.08	5.5	5.5	5.5
4	Aw 3/5 K	6.36	0.814	14.82	6.0	5.8	5.8
5	Aw 1/2 K	10.18	1.211	20.52	6.1	5.7	6.1
6	Aw 3/10 K	6.55	0.789	17.12	6.4	6.1	6.1
7	Aw h/Al	5.21	0.862	17.26	3.6	3.6	5.3
8	AwAl	6.39	0.893	17.60	5.3	5.4	5.8
9	BwO	8.24	1.124	19.96	5.9	7.1	7.1
10	2(BwO)	7.65	1.010	19.27	5.8	7.3	8.0
11	BwK_2	3.22	0.500	15.00	7.1	7.4	7.8
12	BwCa	6.47	0.950	21.34	7.6	7.6	7.8
13	BwAl	13.85	1.954	25.33	7.1	7.4	7.7
14	CwO	5.60	0.683	14.89	4.8	5.6	6.5
15	CwNH	3.71	0.603	14.87	4.3	4.6	4.8
16	CwGC	5.29	0.796	16.21	6.7	7.0	7.8
17	CwGP	4.37	0.640	14.10	7.1	7.0	7.4
18	Cw /1	8.44	1.045	18.54	5.3	5.8	7.2
19	Cw /2	7.72	1.022	16.96	5.8	6.0	6.6
20	Cw /5	6.15	0.928	16.23	6.4	6.4	7.3
21	CwAl	8.20	1.178	20.74	5.4	5.8	7.0

Series 3 was arranged with a view to repeating some of the work previously conducted and likewise to an extension of it. Two plants, wheat and corn, were employed, and the subdivisions of the series may be appropriately designated 3w4, 3w10, 3c4, and 3c10, indicating respectively (3w4) cultures with wheat, solutions renewed every 4 days; (3w10) cultures with wheat, solutions changed every 10 days; (3c4) cultures with corn, solutions changed every 4 days; and (3c10) cultures with corn, solutions changed every 10 days. In this case, therefore, a culture of any plant in a particular solution with a change of solution every 4 days, was duplicated by a similar culture in which the solution was renewed every 10 days.

Sections 3w4 and 3c4 of this series were grown on the rotating table, while sections 3w10 and 3c10 were grown in another green-

TABLE VIII
(Series 3, Wheat)

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH; SOLUTIONS A, B, AND C; RENEWAL AFTER A TEN-DAY INTERVAL

No.	Culture indices	Total gr. wt. (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H		
					Initial	End 1st per.	End 2nd per.
1	AwO	2.55	0.482	12.40	4.8	4.6	4.8
2	Aw 9/10 K	5.04	0.765	15.29	5.3	5.6	6.3
3	Aw 4/5 K	5.50	0.812	14.07	5.5	5.2	5.9
4	Aw 3/5 K	5.52	0.748	13.76	6.0	5.6	6.1
5	Aw 1/2 K	5.53	0.849	12.23	6.1	5.5	6.4
6	Aw 3/10 K	1.06	0.262	10.64	6.4	6.0	6.1
7	Aw h/Al	2.52	0.506	13.61	3.6	3.8	4.9
8	AwAl	4.40	0.668	13.52	5.3	5.5	6.1
9	BwO	7.62	1.079	20.12	5.9	7.6	7.4
10	2(BwO)	7.90	1.094	19.11	5.8	7.6	8.0
11	BwK_2	2.82	0.470	14.42	7.1	8.2	8.5
12	BwCa	5.12	0.789	17.14	7.6	7.8	8.8
13	BwAl	10.30	1.622	26.65	7.1	7.6	8.0
14	CwO	4.95	0.707	14.10	4.8	5.9	6.7
15	CwNH	5.57	0.858	16.15	4.3	4.6	5.3
16	CwGC	5.50	0.846	16.91	6.7	7.6	8.8
17	CwGP	1.70	0.308	10.77	7.1	7.4	7.6
18	Cw /1	7.10	0.995	16.24	5.3	6.2	7.7
19	Cw /2	6.42	0.887	16.62	5.8	6.4	7.6
20	Cw /5	5.50	0.778	16.28	6.4	6.8	7.4
21	CwAl	6.45	0.971	14.67	5.4	6.4	7.4

house, and the cultures were distantly spaced on a lattice table, or bench, constructed with the idea of providing for all cultures uniform circulation, or conditions favoring uniform water loss. The light relations were perhaps somewhat more favorable for the group of cultures renewed at four-day intervals. This series was begun on May 14. During the first two weeks the weather was moderate and cloudy, while during the last week it was bright and warm, perhaps too warm for the best growth of wheat. While the evaporation from a standardized spherical atmometer was low, seldom exceeding 12 gms. per day, during the first two weeks it rose to a maximum of 27.8 and 25.4 as a record for the two houses on May 31.

The modification in the A group of cultures in this series consisted chiefly in the introduction of K_2HPO_4 as a source of part

TABLE IX
(Series 3, Corn)

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH; SOLUTIONS A, B, AND C; RENEWAL EVERY FOUR DAYS

No.	Culture indices	Total gr. wt. (gms.)	Gr. wt. of roots (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H		
						Initial	End 2nd per.	End 4th per.
1	Ac h/1	9.55	1.70	1.422	16.78	3.4	3.4	5.2
2	Ac h/2	32.10	7.08	3.301	27.92	3.7	4.1	5.6
3	AcO	50.61	12.94	4.716	35.74	4.8	4.8	5.6
4	Ac 9/10 K	33.08	10.24	3.015	26.58	5.3	5.6	5.8
5	Ac 4/5 K	41.12	10.13	3.898	30.50	5.5	5.3	6.2
6	Ac 3/5 K	40.90	9.76	3.820	29.60	6.0	5.6	6.3
7	Ac 1/2 K	40.80	12.98	3.434	29.98	6.1	5.6	6.9
8	Ac 3/10 K	47.20	15.24	3.915	31.75	6.4	6.0	6.9
9	Ac h/Al	16.57	3.82	2.117	19.93	3.6	4.6	5.3
10	AcAl	27.78	7.85	3.288	23.05	5.3	5.2	6.3
11	BcO	36.06	9.65	4.107	29.32	5.9	6.8	6.8
12	2BcO	49.72	13.75	5.159	34.11	5.8	7.1	7.0
13	BcK_2	43.23	12.12	4.290	30.94	7.1	7.0	7.0
14	BcCa	36.41	11.01	4.305	29.78	7.6	7.2	7.2
15	BcAl	37.90	12.45	4.597	32.46	7.1	7.1	5.7
16	CcO	29.02	8.58	2.920	23.95	4.8	5.2	7.2
17	CcNH	20.44	4.68	2.307	22.44	4.3	4.6	4.6
18	CcGC	33.33	9.86	3.210	25.82	6.7	6.8	7.7
19	CcGP	32.82	8.83	3.061	26.57	7.1	6.6	7.8
20	Cc /1	35.83	10.82	3.437	29.01	5.3	5.6	7.8
21	Cc /2	35.32	8.80	3.306	27.84	5.8	6.1	7.6
22	Cc /5	35.70	10.25	3.271	28.18	6.4	6.4	8.0
23	CcAl	41.83	12.33	4.201	29.98	5.4	5.7	7.6

of the potassium and phosphate ions. The stock solution of this salt was made to contain the same number of gram atoms of PO_4 as the stock solution of KH_2PO_4. These solutions were then combined so that the ratios of monobasic to dibasic phosphate were respectively 9: 1, 4: 1, 3: 2, 1: 1, and 3: 7, the culture indices being Ax 9/10 K, Ax 4/5 K, Ax 3/5 K, Ax 1/2 K, and Ax 3/10 K, with the P_H of the solutions affected as indicated in the tables. In culture AxO, another high grade of KH_2PO_4 was employed, and the P_H exponent is higher than the theoretical; while in the Ax h/1 cultures, used especially in the case of corn, the more acid grade is employed. Ac h/2 is intermediate in

TABLE X
(Series 3, Corn)

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH; SOLUTIONS A, B, AND C; RENEWAL AFTER A TEN-DAY INTERVAL

No.	Culture indices	Total gr. wt. (gms.)	Gr. wt. of roots (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H		
						Initial	End 1st per.	End 2nd per.
1	Ac h/1	13.70	3.15	2.080	22.42	3.4	5.1	6.4
2	Ac h/2	18.13	3.83	2.268	23.77	3.7	5.4	6.3
3	AcO	34.42	7.75	3.922	31.65	4.8	4.0	5.2
4	Ac 9/10 K	34.82	9.10	3.282	29.64	5.3	5.6	5.3
5	Ac 4/5 K	34.12	7.72	3.298	27.17	5.5	5.1	5.3
6	Ac 3/5 K	37.62	9.70	3.589	28.07	6.0	5.6	5.4
7	Ac 1/2 K	41.80	11.30	3.784	28.76	6.1	5.6	5.4
8	Ac 3/10 K	37.50	12.56	3.397	27.25	6.4	5.8	6.4
9	Ac h/Al	19.67	4.34	2.491	23.23	3.6	5.2	6.1
10	AcAl	32.29	9.22	3.389	25.70	5.3	5.8	5.3
11	BcO	28.26	8.78	3.366	24.36	5.9	7.2	5.5
12	2BcO	38.04	7.77	4.525	30.22	5.8	7.7	5.3
13	BcK_2	43.00	12.63	4.172	31.83	7.1	8.0	5.8
14	BcCa	29.85	9.20	3.617	26.59	7.6	8.4	7.3
15	BcAl	27.80	7.70	3.271	27.75	7.1	7.0	5.6
16	CcO	30.65	8.40	2.923	24.84	4.8	5.6	7.6
17	CcNH	14.44	3.80	1.994	23.85	4.3	4.6	5.8
18	CcGC	27.50	6.97	2.788	23.94	6.7	7.6	8.4
19	CcGP	32.37	8.90	3.145	23.83	7.1	7.3	8.2
20	Cc /1	31.85	7.20	3.060	26.95	5.3	6.2	7.5
21	Cc /2	34.50	9.35	3.005	29.65	5.8	6.4	7.4
22	Cc /5	38.90	9.25	3.487	29.43	6.4	7.0	7.1
23	CcAl	25.06	5.89	2.699	24.74	5.4	6.4	7.7

acidity between AcO and Ac h/1, with salt proportions the same. Again, to other cultures containing these two grades of phosphates there were added to each culture vessel (and with each renewal) 1 gm. of solid aluminium hydroxide of the highest purity procurable. The latter are designated AxAl and Ax h/1 Al respectively.

In the B group cultures were prepared with the addition of solid aluminium hydroxide (BxAl), as above, also with solid calcium carbonate BxCa, with the addition of a small amount of K_2HPO_4 (BxK_2), and the unmodified solution of double strength [2(BxO)].

The C group of cultures (Livingston-Tottingham medium) is here introduced for the first time. Explanations already given explain the culture indices in this group, except in the following cases: CxNH, CxGC, and CxGP. In the first mentioned one-half the atomic proportion of nitrogen is supplied as $(NH_4)_2SO_4$, and in the last two calcium glycero-phosphate is added to replace monobasic calcium phosphate. In CxGC the atomic proportion of Ca is kept the same as in CxO, while in CxGP it is the phosphorus which is equivalent.

Under the conditions of these experiments the Aw cultures, particularly, were less satisfactory than usual, possibly in large part due to the high temperature prevailing towards the end of the period. The series was discontinued earlier than planned owing to the drying of the leaf tips of wheat and even of some entire plants. On the whole the wheat cultures show many inconsistencies, but despite this also some strikingly interesting results.

Increasing the P_H exponent by means of the dibasic phosphate may under these extreme conditions more than treble or quadruple the growth quantities. It is probably a matter of shifting the sum of conditions from the side of toxicity to that of growth maintenance. Even in the case of the C solution, increasing the P_H exponent is here a factor in promoting growth increase. Under other conditions I have not found this to be true, as will be indicated later.

The addition of aluminium hydroxide is under these conditions distinctly favorable, as seen by comparing the following pairs, AwO and AwAl (also AwO and Aw h/Al), BwO and BwAl, CwO and CwAl. The value of this reagent is doubtless in part due to its action as a buffer.

The temperature was most favorable for the corn cultures, and they exhibited, on the whole, an unusually vigorous growth. The green weight determinations are an accurate indication of growth extent but not of appearance. The B group was dark green in color, with heavy purplish stems, while both the A and C groups were strongly chlorotic. Chlorosis was much intensified during the last week of growth, and it seemed probable that if these cultures were longer maintained, a considerable reduction in the growth rate would occur. It is assumed, how-

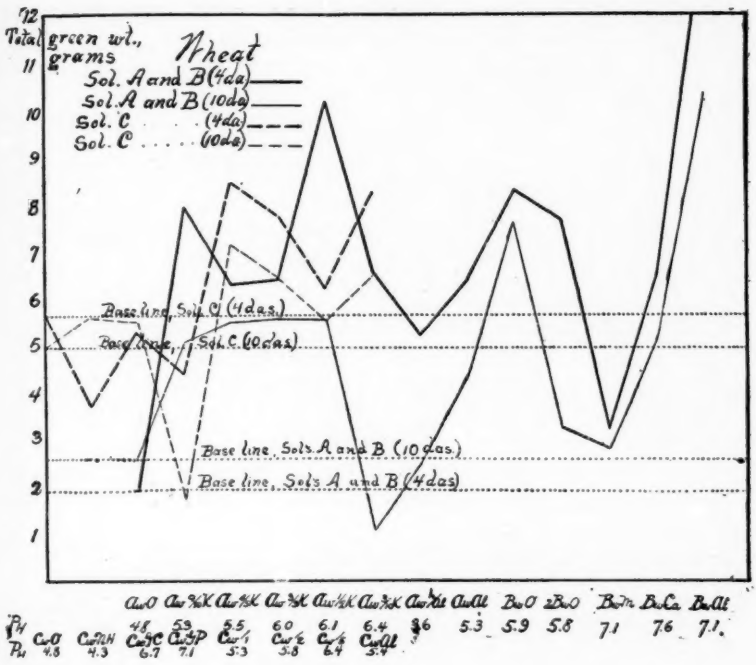

Fig. 3. Total green weight of wheat in solutions A, B, and C, with modifications effected largely by combinations of phosphates or by the introduction of "insoluble" buffers. Intervals between the renewal of solutions were 4 and 10 days.

ever, that in these cases the chlorosis may be related to inadequate iron supply, and not to faulty proportions of the main salt constituents.

The Ac h/1, Ac h/2 and Ac h/Al cultures all show the injurious effect of high acidity; but with the initial value of P_H 4.8 the best growth in the one lot is in the A solution, though practically approached in the double strength of the B solution [2(BcO)]. Aside from the considerable variation in cultures differing only slightly in composition or in P_H, the chief point of interest is the depressing action of the ammonium salt in both lots (BcNH).

The curves, figs. 3 (wheat) and 4 (corn), exhibit diagrammatically the data above discussed, and require no explanation, further than to point out that the results with solution C appear

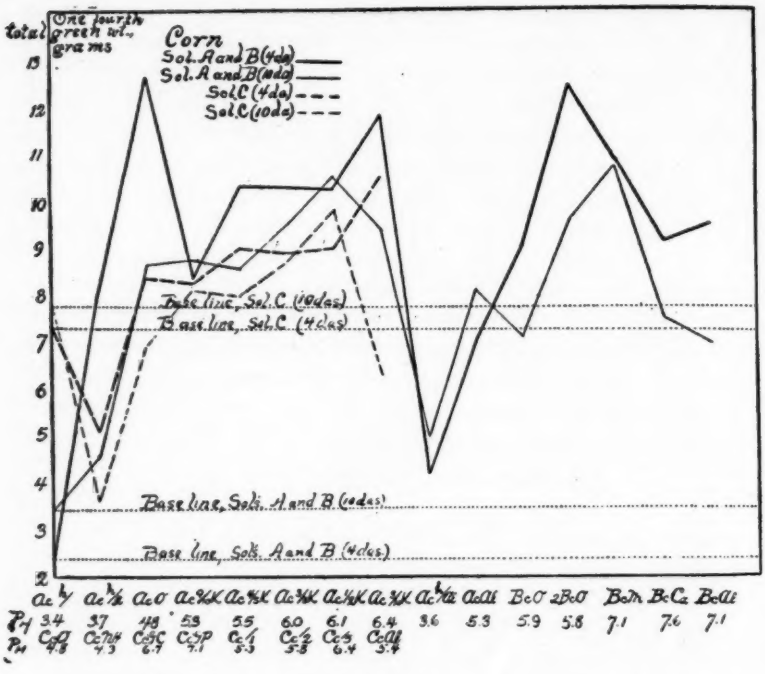
One fourth total green wt., grams
Corn
Sol. A and B (4da)
Sol. A and B (10da)
Sol C (4da)
Sol C (10da)
Base line, Sol. C (10days)
Base line, Sol. C (4days)
Base line, Sols. A and B (10days)
Base line, Sols. A and B (4days)
Ac ½ Ac ½ Ac 0 Ac ½K Ac ½K Ac ½K Ac ½K Ac ½K Ac ½a Ac all Bc 0 2Bc 0 Bc ½ Bc Ca Bc all
P4 3.4 3.7 4.8 5.8 5.5 6.0 6.1 6.4 3.6 5.3 5.9 5.8 7.1 7.6 7.1
P4 Ca0 Ca½ Ca0 Ca½ Ca½ Ca½ Ca½ Ca½
4.8 4.3 6.7 7.1 5.3 5.8 6.4 5.4

Fig. 4. One-fourth total green weight of corn in solutions A, B, and C. See further explanation under fig. 3.

in the same region of fig. 3 as solution A merely because it was necessary to do this in order to present all the data in a single figure.

In order to include solution C again in the tests, and at the same time to change somewhat the range of hydrogen ion concentrations in the A and B solutions, as well as to repeat the former work, a more extensive series of experiments was arranged with wheat, corn, and peas as indicated by the results in tables XI–XIX. Meanwhile, it had been determined from preliminary experiments that nutrient solutions of diverse constitution seem to be considerably influenced by the conditions under which the cultures were grown. There was, therefore, introduced into this series three sets of conditions. On account of the fact that other experiments were under way in the green-

TABLE XI

(Series 4, I—Wheat [moist, high temperature])

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH

No.	Culture indices	Total gr. wt. (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H	
					Initial	After gr.
1	AwO	4.19	.491	30.71	3.4	4.0
2	Aw /5	4.10	.400	26.77	4.6	5.3
3	Aw /10	3.78	.399	27.00	5.8	4.4
4	Aw 1/	2.60	.338	24.63	3.35	4.4
5	Aw 3/	2.22	.328	22.08	3.15	3.6
6	AwNa	3.83	.468	30.10	3.45	3.8
7	AwM	4.65	.485	29.23	5.4	4.0
8	BwO	5.22	.469	32.96	6.6	7.2
9	Bw /5	4.90	.466	32.84	7.4	7.6
10	Bw /10	4.65	.424	32.04	8.6	8.2
11	Bw 1/	5.38	.491	32.06	6.4	7.2
12	Bw 5/	5.35	.472	31.33	3.2	6.8
13	Bw 10/	.95	.177	12.26	2.8	3.8
14	BwK_2 /10	5.13	.466	35.55	7.0	7.2
15	BwCa	5.70	.539	34.23	7.3	6.6
16	CwO	4.82	.465	29.12	4.2	6.4
17	Cw /5	4.17	.401	24.78	6.4	7.0
18	Cw /20	2.25	.301	18.85	7.6	7.2
19	Cw 1/	4.05	.403	26.50	3.6	6.4
20	Cw 5/	2.47	.296	20.85	3.2	5.2
21	CwNa	4.50	.441	28.72	4.2	6.6

houses at the same time no particular effort was made to control accurately the conditions of growth. The following general conditions were decided upon: (I) Moist high temperature, (II) moist low temperature, and (III) dry high temperature. These conditions are relative, of course, and all supported good growth. "Moist" in the sense here used simply means that by frequent sprinkling of walls and floors the humidity was raised above that of the usual greenhouse compartment. However, in the case of those cultures placed under conditions of high moisture and high temperature there was also necessary a slight degree of shade. This caused the plants to grow up rather quickly. As a result of this rapid growth in this section of the series it was determined to take down all cultures after about 15 days except those placed under the conditions referred to as moist, low

TABLE XII

(Series 4, II—Wheat [moist, low temperature])

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH

No.	Culture indices	Total gr. wt. (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H	
					Initial	After gr.
1	AwO	3.72	.681	16.81	3.4	4.0
2	Aw /5	10.87	1.373	22.90	4.6	6.0
3	Aw /10	8.85	1.087	21.40	5.8	6.4
4	Aw 1/	4.42	.736	16.75	3.35	4.0
5	Aw 3/	3.27	.579	16.23	3.15	3.4–3.5
6	AwNa	6.80	.862	19.28	3.45	5.8
7	AwM	11.24	1.323	24.20	5.4	6.6
8	BwO	10.92	1.035	20.75	6.6	7.0
9	Bw /5	8.40	.912	19.27	7.4	7.6
10	Bw /10	9.02	.924	22.12	8.6	8.1
11	Bw 1/	7.37	.908	20.69	6.4	7.1
12	Bw 5/	9.17	.910	20.06	3.2	7.2
13	Bw 10/	.90	.209	8.80	2.8	3.6
14	BwK_2 /10	8.82	.984	19.46	7.0	7.5
15	BwCa	11.64	1.354	20.51	7.3	7.4
16	CwO	6.75	.870	18.54	4.2	7.7
17	Cw /5	6.77	.826	16.93	6.4	7.8
18	Cw /10	5.60	.681	12.85	7.6	8.6
19	Cw 1/	7.22	.915	18.90	3.6	7.7
20	Cw 5/	7.55	.916	18.17	3.2	7.5
21	CwNa	7.75	.853	18.78	4.2	7.8

temperature. On account of conditions prevailing at the time, sections I and III were actually maintained from 16 to 18 days. The plants in section II were permitted to run from 25 to 27 days. This was an extreme test of the tolerance of these solutions, and no change of the nutrient medium was made in any culture throughout the entire period of growth. The water lost by transpiration was, however, replaced from day to day.

A general survey of the blocks of cultures represented by solutions A, B, and C gives evidence that under conditions of rather moist air and high temperature (table XI) wheat grown without change of solution for 18 days exhibits less striking differences in the maximum growth quantities than may be seen in the earlier series of cultures. Nevertheless, the highest value, whether of total green weight or of dry weight, is found in culture

TABLE XIII

(Series 4, III—Wheat [dry, high temperature])

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH

No.	Culture indices	Total gr. wt. (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H	
					Initial	After gr.
1	AwO	2.77	.395	19.70	3.4	4.8
2	Aw /5	5.50	.582	26.23	4.6	5.8
3	Aw /10	5.61	.601	24.90	5.8	6.1
4	Aw 1/	1.78	.287	17.14	3.35	3.4
5	Aw 3/	1.55	.248	16.33	3.15	3.4
6	AwNa	3.66	.441	23.70	3.45	3.6
7	AwM	3.35	.390	21.89	5.4	5.0
8	BwO	7.43	.658	26.54	6.6	7.2
9	Bw /5	7.25	.733	30.97	7.4	7.6
10	Bw /10	5.64	.493	22.37	8.6	8.6
11	Bw 1/	4.72	.554	24.38	6.4	8.6
12	Bw 5/	5.42	.566	25.19	3.2	7.3
13	Bw 10/	.17	.092	6.67	2.8	2.9
14	$BwK_2/10$	6.40	.615	26.58	7.0	7.9
15	BwCa	7.39	.765	28.62	7.3	8.2
16	CwO	4.49	.510	21.60	4.2	7.2
17	Cw /5	4.97	.576	23.43	6.4	7.6
18	Cw /20	3.32	.450	20.20	7.6	8.2
19	Cw 1/	4.80	.491	23.66	3.6	7.0
20	Cw 5/	2.00	.298	19.02	3.2	5.0
21	CwNa	4.50	.522	24.00	4.2	7.4

BwCa. Several other cultures in the B block, notably Bw 1/ and Bw 3/, exceed slightly all those of the C block by either of the 2 important criteria, that is, green weight or dry weight. Under these conditions the tolerance of high hydrogen ion concentration is generally marked, as shown in cultures AwO, AwNa, Bw 5/, and Cw 1/. Likewise the range of tolerance and of strong growth is considerable.

In section II of this series with wheat (table XII) it is the inference from the data that when grown for a longer period (25 days) under cooler conditions wheat is less resistant to high hydrogen ion concentration. This is shown in part by the fact that the 4 cultures yielding highest in green weight are BwCa, AwM, BwO, and Aw /5. There is, however, no striking falling off, on the whole, as hydrogen ion concentration increases to

TABLE XIV

(Series 4, I—Corn [moist, high temperature])

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH

No.	Culture indices	Total gr. wt. (gms.)	Gr. wt. of roots (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H	
						Initial	After gr.
1	AcO	19.05	4.42	.593	34.72	3.4	4.2
2	Ac /5	27.32	6.80	1.786	40.20	4.6	4.6
3	Ac /10	18.85	3.87	.305	38.58	5.8	6.0
4	Ac 1/	14.83	4.01	1.136	30.48	3.35	6.3
5	Ac 3/	9.45	3.28	1.129	29.23	3.15	6.4
6	AcNa	6.44	3.09	.973	22.77	3.45	6.6
7	AcM	24.02	4.80	1.615	38.24	5.4	6.5
8	BcO	22.31	4.93	1.781	38.63	6.6	5.2
9	Bc /5	19.77	2.44	1.452	39.65	7.4	6.4
10	Bc /10	21.12	4.97	1.538	39.64	8.6	6.1
11	Bc 1/	20.93	3.81	1.726	40.85	6.4	5.6
12	Bc 5/	21.55	4.33	1.724	41.25	3.2	4.4
13	Bc 10/	9.02	2.38	.936	22.71	2.8	7.1
14	BcK_2 /10	25.56	4.44	1.770	43.46	7.0	5.2
15	BcCa	19.02	2.70	1.572	43.40	7.3	7.2
16	CcO	20.02	4.70	1.396	27.26	4.2	7.7
17	Cc /5	16.11	3.28	1.105	26.72	6.4	7.4
18	Cc /20	15.81	4.81	1.207	22.18	7.6	7.9
19	Cc 1/	19.81	4.88	1.341	33.56	3.6	7.6
20	Cc 5/	26.72	6.67	1.780	31.02	3.2	7.6
21	CcNa	18.81	3.94	1.165	30.62	4.2	7.7

about P_H 3.2, although there is not the consistency which might be expected between cultures AwO, Cw 1/, Cw 5/, and Bw 5/.

Where the conditions involved a relatively dry atmosphere and a high greenhouse temperature the response of the organism to the different culture solutions is of special interest. The three cultures with highest green weight yields are all in the B block, namely, BwO, BwCa, and Bw /5. These are so far ahead of the cultures in the A and C blocks, irrespective of hydrogen ion concentration within the usual range, as to leave no doubt whatever that for continued cultivation in the same solution for a period of 18 days the B solution is decidedly the most favorable culture medium. In this case too we have as definite an indication as has been afforded as to the limitation imposed by hydrogen ion concentrations. With an exponent

TABLE XV

(Series 4, II—Corn [moist, low temperature])

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH

No.	Culture indices	Total gr. wt. (gms.)	Gr. wt. of roots (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H	
						Initial	After gr.
1	AcO	8.77	3.25	1.007	15.83	3.4	5.6
2	Ac /5	22.81	8.44	1.703	22.98	4.6	6.0
3	Ac /10	23.10	8.94	1.521	22.78	5.8	6.2
4	Ac 1/	21.97	6.94	1.933	21.75	3.35	5.8
5	Ac 3/	11.28	2.82	1.303	15.65	3.15	6.0
6	AcNa	19.30	7.91	1.744	20.56	3.45	6.0
7	AcM	27.56	10.69	2.078	22.72	5.4	6.0
8	BcO	18.90	7.50	1.828	20.64	6.6	6.4
9	Bc /5	31.90	14.50	2.508	23.51	7.4	5.9
10	Bc /10	28.63	14.54	2.213	21.84	8.6	7.0
11	Bc 1/	23.61	7.74	2.423	24.14	6.4	4.8
12	Bc 5/	27.80	10.60	2.464	23.44	3.2	6.0
13	Bc 10/	15.50	6.20	1.366	16.19	2.8	5.6
14	BcK_2 /10	36.30	6.44	2.950	23.50	7.0	5.7
15	BcCa	26.03	10.11	2.699	23.57	7.3	7.4
16	CcO	34.60	11.02	2.763	24.66	4.2	7.6
17	Cc /5	28.47	9.72	2.350	23.59	6.4	7.6
18	Cc /20	20.90	6.75	1.868	20.10	7.6	8.6
19	Cc 1/	23.52	7.55	1.966	23.40	3.6	7.6
20	Cc 5/	21.22	6.70	1.687	22.15	3.2	7.6
21	CcNa	24.60	9.07	2.090	19.74	4.2	7.6

below P_H 4 there is definite diminution of growth in every instance, except Bw 5/. AwM seems to be distinctly out of harmony in this group. The suggestion from culture Bw /10 is that under these climatic conditions a relatively low degree of alkalinity, probably about P_H 8, would represent the limit for most favorable growth in a solution thus constituted.

The results with corn grown in a moist atmosphere at a high temperature are perhaps more erratic than in any other section of the work. At least with the data at hand it is extremely difficult to interpret these results. It would appear that the best growth is in culture Ac /5, and it is closely followed by Cc 5/, the last solution being close to the usual limit of growth in respect to hydrogen ion concentration. In both these cases the increase in the growth over that in BcK_2/10, AcM, and BcO is

TABLE XVI

(Series 4, III—Corn [dry, high temperature])

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH

No.	Culture indices	Total gr. wt. (gms.)	Gr. wt. of roots (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H	
						Initial	After gr.
1	AcO	24.43	8.59	1.812	24.35	3.4	5.9
2	Ac /5	25.90	7.94	1.751	22.81	4.6	6.0
3	Ac /10	17.22	5.28	1.360	27.64	5.8	6.2
4	Ac 1/	21.80	5.60	1.667	31.24	3.35	4.0
5	Ac 3/	17.49	4.61	1.405	23.65	3.15	4.6
6	AcNa	16.00	4.70	1.231	27.17	3.45	5.6
7	AcM	30.41	9.61	2.014	32.26	5.4	6.2
8	BcO	19.89	6.30	1.604	27.07	6.6	4.0
9	Bc /5	20.89	7.11	1.756	29.80	7.4	6.8
10	Bc /10	20.90	5.85	1.810	30.41	8.6	6.8
11	Bc 1/	23.00	9.25	1.637	27.75	6.4	5.0
12	Bc 5/	20.02	7.70	1.442	29.59	3.2	4.2
13	Bc 10/	1.64	.43	.285	5.11	2.8	3.4
14	BcK_2 /10	24.52	9.89	1.665	31.33	7.0	5.2
15	BcCa	23.44	8.81	1.867	32.53	7.3	7.8
16	CcO	16.62	4.97	1.125	25.67	4.2	7.8
17	Cc /5	18.52	5.20	1.180	32.71	6.4	8.0
18	Cc /20	11.28	4.06	.835	14.88	7.6	8.0
19	Cc 1/	20.56	5.65	1.407	29.97	3.6	7.9
20	Cc 5/	8.63	1.61	.856	20.75	3.2	7.6
21	CcNa	15.54	5.57	1.122	25.31	4.2	7.8

largely an increase in the growth of roots. It is perhaps possible that the partial shade of this series referred to above has been a factor in the irregularities which prevail throughout. There is no basis on which to explain the growth in AcNa. Turning, however, to table XV, indicating the results with corn grown at low temperature and higher humidity the data are notably different from the preceding. In this case the most favorable medium is culture $BcK_2/10$, rather closely followed by CcO and less closely by Bc5/. In this section it is also notable that the maximum root growth occurs over a range of hydrogen ion concentration from 4.2 to 8.6. Exclusive of culture CcO the higher growth quantities are obtained for the different solutions at relatively low hydrogen ion concentration, that is, with media approaching neutrality more or less. This is particularly ob-

TABLE XVII

(Series 4, I—Peas [moist, high temperature])

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH

No.	Culture indices	Total gr. wt. (gms.)	Gr. wt. of roots (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H	
						Initial	After gr.
1	ApO	12.53	4.15	1.010	28.46	3.4	3.5
2	Ap /5	13.82	4.15	1.059	31.05	4.6	4.2
3	Ap /10	13.92	3.87	1.062	13.25	5.8	5.8
4	Ap 1/	11.55	3.42	.937	28.00	3.35	4.7
5	Ap 3/	11.93	3.17	.990	29.68	3.15	5.8
6	ApNa	12.56	3.93	1.024	31.42	3.45	4.6
7	ApM	12.81	3.93	.966	30.00	5.4	4.6
8	BpO	10.60	3.02	.839	25.87	6.6	6.4
9	Bp /5	13.17	3.64	.957	31.97	7.4	7.5
10	Bp /10	9.80	3.11	.746	32.47	8.6	7.6
11	Bp 1/	11.17	3.47	.809	23.37	6.4	7.0
12	Bp 5/	11.12	2.89	.842	26.22	3.2	5.8
13	Bp 10/	7.59	2.21	.623	23.29	2.8	4.8
14	BpK_2 /10	12.30	3.61	.936	26.62	7.0	7.3
15	BpCa	12.55	3.50	.937	29.57	7.3	7.6
16	CpO	12.44	3.39	.974	25.34	4.2	5.2
17	Cp /5	8.19	2.16	.657	21.66	6.4	6.2
18	Cp /20	10.52	2.93	.928	22.37	7.6	7.5
19	Cp 1/	12.50	3.50	.982	26.02	3.6	5.0
20	Cp 5/	6.93	2.01	.605	21.36	3.2	4.2
21	CpNa	10.72	3.06	.923	22.35	4.2	5.4

servable in cultures Ac /10, Bc /5, Bc /10, BcCa, and Cc /5. Corn grown under conditions of high temperature and low humidity exhibits a maximum in AcM followed respectively by BcK_2/10, Ac /5, BcCa, and Bc 1/. An examination of block A would seem to indicate that P_H 3.4 to 4.6 is entirely favorable in this medium, but there is a striking difference between Ac/10 and AcM, which is not readily explainable.

In table XVII it may be noted again that slight variations in the culture medium do not materially affect the growth of peas when grown under the conditions there indicated. The A solution with the addition of alkali yields, it is true, the maximum growth quantities, but these quantities are only slightly in excess of those obtained with the same solution unmodified or of several cultures in the B block, notably Bp /5, BpK_2/10, and

TABLE XVIII

(Series 4, II—Peas [moist, low temperature])

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH

No.	Culture indices	Total gr. wt. (gms.)	Gr. wt. of roots (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H	
						Initial	After gr.
1	ApO	17.72	6.55	1.772	19.07	3.4	5.5–5.6
2	Ap /5	25.71	12.17	2.290	18.06	4.6	6.0
3	Ap /10	24.26	9.72	2.299	20.00	5.8	5.9–6.0
4	Ap 1/	21.05	9.00	1.980	17.77	3.35	5 9
5	Ap 3/	22.52	9.44	2.183	19.18	3.15	5.6
6	ApNa	20.69	8.52	.889	17.61	3.45	5.7
7	ApM	25.65	11.20	2.310	19.87	5.4	
8	BpO	13.87	8.26	1.400	15.82	6.6	6.1–6.2
9	Bp /5	13.65	5.18	1.308	14.74	7.4	7.1
10	Bp /10	18.57	7.54	1.704	15.63	8.6	7.6
11	Bp 1/	16.93	6.61	1.590	17.38	6.4	6.0
12	Bp 5/	16.14	7.59	1.378	13.30	3.2	6.0
13	Bp 10/	9.55	3.88	.881	10.98	2.8	5.5
14	BpK_2 /10	18.11	8.90	1.455	16.26	7.0	6.2
15	BpCa	20.17	8.23	1.944	19.28	7.3	7.4
16	CpO	16.44	6.44	1.650	13.85	4.2	7.2
17	Cp /5	16.31	6.10	1.762	13.62	6.4	7.8
18	Cp /20	12.32	4.54	1.406	10.82	7.6	7.7
19	Cp 1/	16.36	6.95	1.599	13.61	3.6	7.2
20	Cp 5/	17.62	7.06	1.760	13.22	3.2	6.4
21	CpNa	16.54	5.62	1.711	17.38	4.2	7.0

BpCa, as also by two cultures in the C block, namely CpO and Cp 1/.

Wider differences are found in the case of peas grown at lower temperature in more humid air, but the maximum growth occurs in the A block, especially with the addition of a small amount of alkali (Ap /5) and in the ApM culture, containing both monobasic and dibasic phosphates. No culture in the B block approaches the values referred to, and the same is true of the C cultures. Grown 16 days under dry conditions at a high temperature the yield of the various cultures is much reduced, and the relative values of the culture media do not remain the same as before. In this instance the B block exhibits the highest yields, especially cultures BpCa and BpK_2/10. The next higher yield is found in Ap /10. In the A block the effect of high

TABLE XIX

(Series 4, III—Peas [dry, high temperature])

SALT REQUIREMENTS AND H-ION CONCENTRATION IN RELATION TO GROWTH

No.	Culture indices	Total gr. wt. (gms.)	Gr. wt. of roots (gms.)	Total dry wt. (gms.)	Greatest length (cm.)	P_H	
						Initial	After gr.
1	ApO	7.42	3.05	.952	23.68	3.4	4.6
2	Ap /5	10.62	3.55	1.026	24.63	4.6	5.0
3	Ap /10	13.95	4.49	1.161	27.63	5.8	5.4
4	Ap 1/	3.20	1.10	.642	11.82	3.35	5.3
5	Ap 3/	6.78	2.47	.796	18.84	3.15	5.0
6	ApNa	11.22	3.60	.945	25.33	3.45	4.4
7	ApM	10.00	3.89	1.095	23.57	5.4	5.6
8	BpO	12.79	4.37	1.023	24.06	6.6	4.5
9	Bp /5	12.97	4.15	1.059	25.96	7.4	7.3
10	Bp /10	12.90	4.12	1.027	26.05	8.6	7.4
11	Bp 1/	8.64	2.15	.877	21.11	6.4	7.0
12	Bp 5/	11.95	4.27	.948	24.23	3.2	6.1
13	Bp 10/	2.97	0.75	.432	9.95	2.8	3.0
14	BpK_2 /10	14.32	4.90	1.130	27.37	7.0	7.3
15	BpCa	14.44	4.92	1.233	26.33	7.3	7.8
16	CpO	6.60	2.10	.929	16.91	4.2	6.0
17	Cp /5	11.65	3.50	1.046	21.43	6.4	6.6
18	Cp /20	5.11	1.22	.760	15.40	7.6	7.6
19	Cp 1/	10.97	3.45	.977	21.28	3.6	5.4
20	Cp 5/	5.40	1.25	.636	15.54	3.2	5.8
21	CpNa	8.75	2.45	.966	18.99	4.2	6.0

hydrogen ion concentration is marked. In general, as the conditions of evaporation are intensified it would appear that somewhat more favorable results are obtained with this plant when the hydrogen ion concentration does not approach too closely the acid limit.

It is clear, however, that the preparation of nutrient solutions with acid phosphate—even though of high grade—may mean, and often does mean, a hydrogen ion concentration either perilously near the critical region for growth, or actually inhibiting growth. This is particularly true for wheat, and it may be true for corn and other crops under any conditions which may accentuate acid injury. Pronounced diminution in growth may occur as the P_H exponent is progressively diminished from about 4.5.

For each plant in the preceding series a separate set of curves (figs. 5, 6, and 7) has been prepared; and in each figure there are

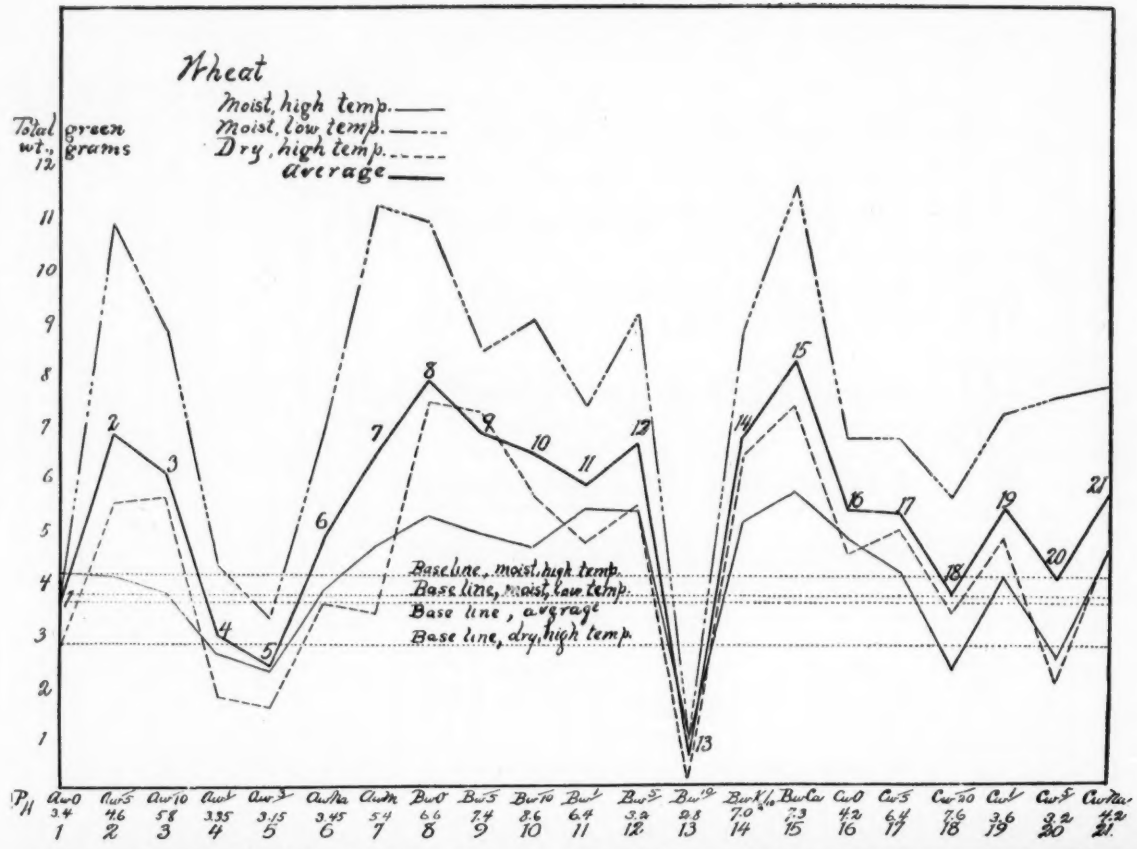

Fig. 5. Total green weight of wheat in solutions A, B, and C, with variations and under somewhat diverse external conditions, as indicated in connection with the curves.

Fig. 5. Total green weight of wheat in solutions A, B, and C, with variations and under somewhat diverse external conditions, as indicated in connection with the curves.

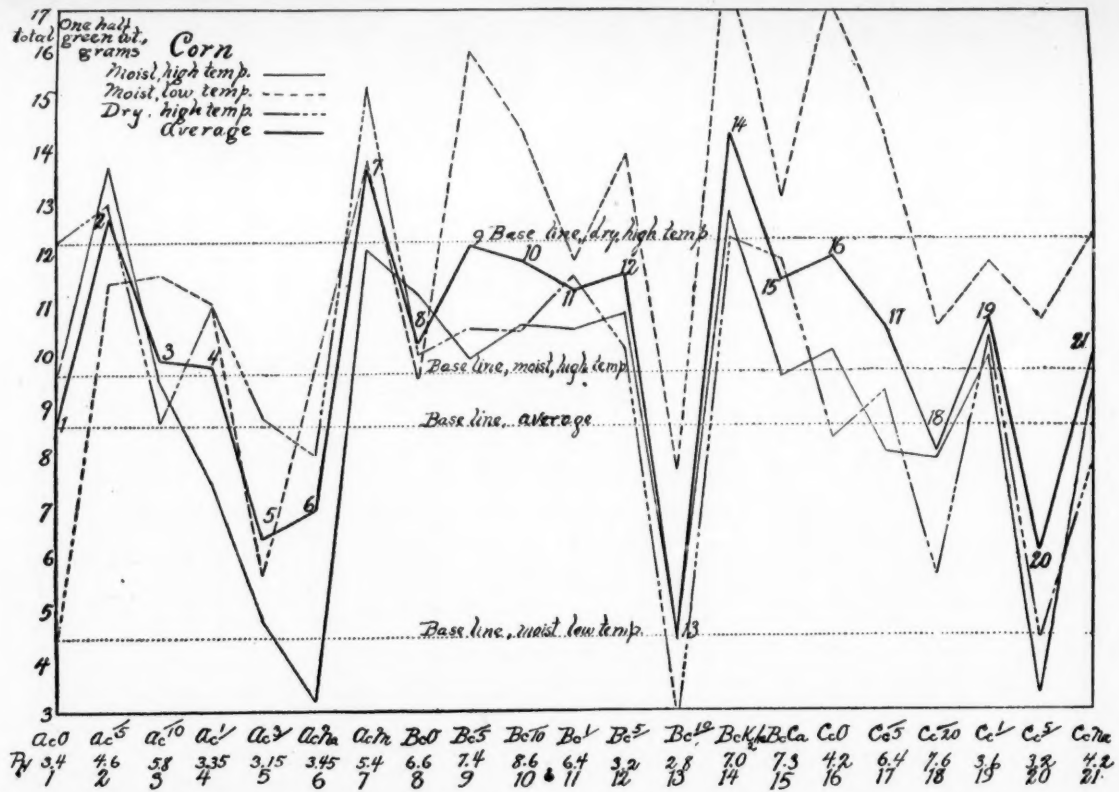
One half total green wt., grams
Corn
Moist, high temp.
Moist, low temp.
Dry, high temp.
Average
Base line, dry, high temp.
Base line, moist, high temp.
Base line, average
Base line, moist, low temp.
Ac0 Ac5 Ac10 Ac1 Ac2 Ac3 Ac4 Bc0 Bc5 Bc10 Bc1 Bc2 Bc3 Bc4 BcCa Cc0 Cc5 Cc10 Cc1 Cc2 Cc3
Pr 3.4 4.6 5.8 3.35 3.15 3.45 5.4 6.6 7.4 8.6 6.4 3.2 2.8 7.0 7.3 4.2 6.4 7.6 3.6 3.2 4.2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Fig. 6. One-half total green weight of corn in solutions A, B, and C, with variations and under somewhat diverse external conditions, as indicated in connection with the curves.

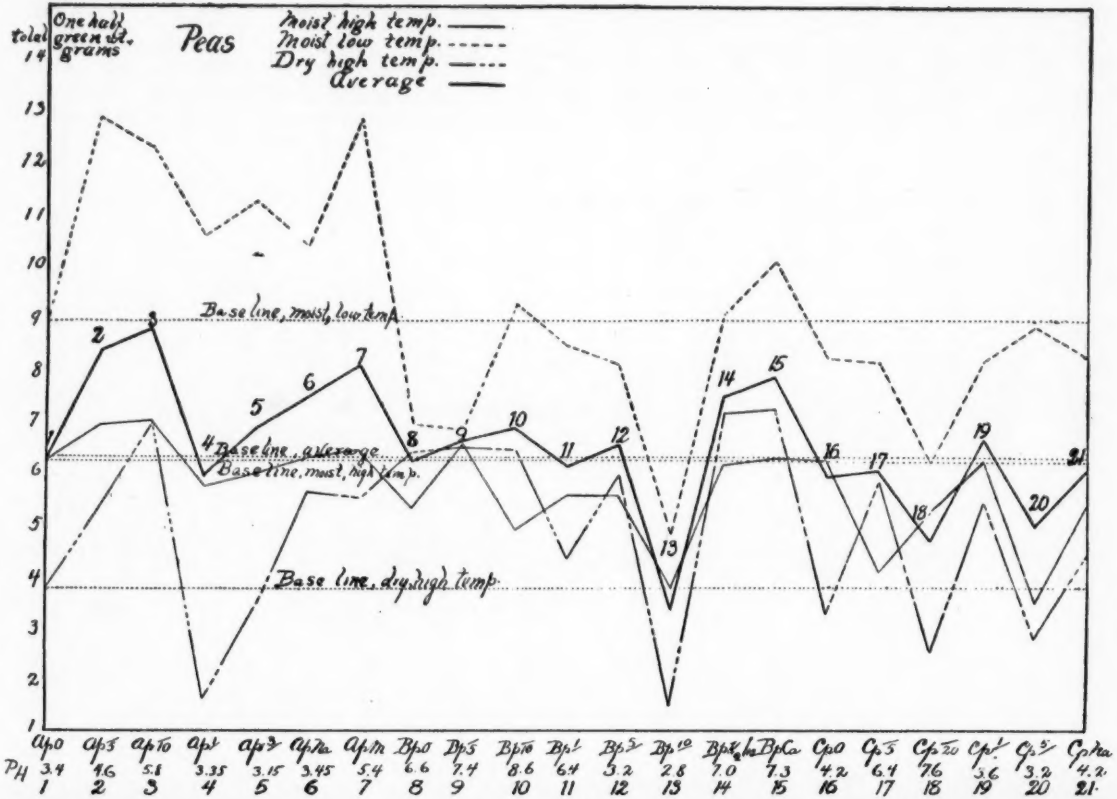
One half total green wt. grams
Peas
Moist high temp.
Moist low temp.
Dry high temp.
Average
Base line, moist, low temp.
Base line, average
Base line, moist, high temp.
Base line, dry, high temp.
Ap0 Ap5 Ap10 Ap1 Ap2 Ap4a Ap4b Bp0 Bp5 Bp10 Bp1 Bp2 Bp10 Bp4a,b Bp4c Cp0 Cp5 Cp20 Cp1 Cp2 Cp4a
pH 3.4 4.6 5.8 3.35 3.15 3.45 5.4 6.6 7.4 8.6 6.4 3.2 2.8 7.0 7.3 4.2 6.4 7.6 5.6 3.2 4.2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Fig. 7. One-half total green weight of Canada field peas in solutions A, B, and C, with variations and under somewhat diverse external conditions, as indicated in connection with the curves.

3 curves representing the results under the 3 combinations of conditions, as well as a fourth curve exhibiting the average of the 3 others.

Fig. 7. One-half total green weight of Canada field peas in solutions A, B, and C, with variations and under somewhat diverse external conditions, as indicated in connection with the curves.

General Discussion

Hoagland ('17) determined the effect of H- and OH-ion concentration on the growth of barley seedlings in an incomplete nutrient solution, omitting calcium, magnesium, and iron,—employing therefore only phosphates of potassium in some series and in others adding to these small proportions of sodium salts, including nitrate. The H-ion adjustments were made on the alkaline side with K_3PO_4 and K_2HPO_4; on the acid side there was used KH_2PO_4, supplemented in one case by H_3PO_4 and by the dibasic salt. As a result of numerous experiments he finds that a concentration of OH-ion greater than 1.8×10^{-6} was injurious, and it was extremely toxic when the concentration reached 2.5×10^{-5}. A concentration of H-ion of $.7\times10^{-5}$ was favorable to growth while $.3\times10^{-3}$ was very toxic. In these solutions there was, of course, opportunity for antagonistic effects, and since the solutions were unbalanced, the injurious effect of the potassium or sodium ions or both would require consideration.

Some of the complicated effects resulting from the addition of salts to toxic acid and alkaline solutions, especially in respect to the water relations of plants, have been dealt with by Dachnowski ('14); but inasmuch as the constituents of nutrient solutions were not involved either in control experiments or otherwise the data are scarcely applicable here.

It is difficult, if not impossible, to attempt a comparison of the toxic action of H-ions and OH-ions from the dissocation respectively of mineral acids and such hydroxides as those of sodium and potassium in distilled water with the toxic effects produced by the same ions in a culture solution containing diverse other ions, especially the cations of the salts usually employed. In the latter solutions antagonistic effects, dependent in part upon specific relations of the plant employed, must to a certain degree obscure the magnitude of the effects. It is of interest to note, however, that Kahlenberg and True ('96) found that roots of *Lupinus albus* just lived in n/6400 HCl. Nevertheless, after 5

days in n/25,600 HCl the growth rate was but little more than one-half that of similar roots in distilled water. Thus we may assume that a hydrogen ion concentration approximately P_H 4 depressed the root growth of this plant. The same seedlings survived n/400 potassium hydroxide during 24 hours, but with this reagent no growth measurements after an interval of several days, comparable to those with HCl, were made. The results of Heald ('96) also show that while roots of *Pisum sativum* survived n/6400 HCl, and those of *Zea Mays* n/1600, still in the former the growth rate was low in n/12,800 and for corn in n/3200, during an interval of 48 hours. No comparisons with distilled water were included.

Loew ('03), working with the seedlings of *Zea Mays*, and Miyake ('14), using *Oryza sativa*, have both shown a relatively greater resistance of these plants to alkali than to acid, all of which emphasizes the importance of devoting special consideration to the initial acidity of the culture solution.

Respecting the reaction of soils, there is considerable evidence indicating that acidity alone is not necessarily a limiting factor in the growth of many crops. With a method considered adequately accurate as applied to field conditions, Gillespie ('16) has examined air-dried samples of 22 crop soils and found the P_H exponent to vary from 4.55 to 7.1 in the case of 18 soils from Maine, Maryland, and Virginia, and a variation of from 8.1 to 8.7 with 4 soils from Utah and Montana. It is also reported by Gillespie and Hurst ('18) that the highest acidity (P_H 4.5) was not in the least injurious to potato culture in Caribou and Washburn loams, the two main potato-soil types in the region in which they worked.

Plummer ('18), employing the soil-suspension method, examined 68 samples of a variety of soil types of humid regions, especially of the southern states. Untreated sandy loam or clay soils exhibited a range of acidity $.1\times10^{-3}$ to 1×10^{-6}, while peat gave in one instance a hydrogen ion concentration $.2\times10^{-1}$. Evidence was gained to the effect that the surface film emphasizes the direction of the reaction, that is, in acid soils the surface film is more acid and in alkaline soils it is more alkaline. This is scarcely in corroboration of certain work of Sharp and Hoagland ('16).

In general, when the reactions of the culture solutions (such as have been employed in this work) are strongly acid, the contact with plant roots effects a change towards neutrality. The extent and rapidity of this change, however, depend somewhat upon the crop, and especially upon the composition of the culture solution. It may be noted, for instance, from tables XI–XIX that solution A never became neutral, while solution C was changed, in the extreme case, to P_H 8.6. On the other hand, it is not necessarily true that alkaline culture solutions tend to become acid, as may be seen in the case of solution C. Solution B, normally near the neutral point, may be shifted slightly in either direction.

Impelled in part by the general experience of others in field work, indicating a general tendency of cultivated soils to become acid, Breazeale and Le Clerc ('12) undertook solution-culture experiments to determine "the effect of the reaction of the culture medium on the growth of wheat seedlings and particularly on the development of the root," with a view to a possible explanation of the results obtained in practical agriculture. They regard the acid tendency as due primarily to the decay of organic matter and secondarily to the selective action of the root; the last mentioned only they proposed to investigate. Their experiments were chiefly with certain salts, particularly KCl, K_2SO_4, and $NaNO_3$, used singly and each in combination with solid $CaCO_3$. According to their results greater absorption of the K ion, when the potassium salts are used, caused the solution to become acid; while in the case of the sodium salt, the greater absorption of the NO_3 ion tended to produce alkalinity. The addition of calcium carbonate precludes the development of acid with the potassium salts. In the toxic action reported no account is taken of the lack of physiological balance in the solutions lacking Ca, and no experiments were made with KNO_3. Moreover, only a titration method (consistent with the general usage at that time) was employed in determining acidity and alkalinity.

Some time previous to this Hartwell and Pember ('07) emphasized the "marked property of the seedlings [wheat, rye, barley, and oats] of rendering the nutrient solutions alkaline—," insufficient, however, to cause precipitation. Similar observations are numerous in the literature.

Hoagland and his associates ('17, '18, '19) in a series of articles have pointed out some misinterpretations and discrepancies in the earlier work, and among other things have shown that with certain proportions of salts in solution cultures and in sand cultures having an initial acid reaction, this reaction was changed with the growth of the crops until it was approximately neutral. In certain cultures with an initial reaction approximately neutral, plants were grown to maturity without change of solution, and with the reaction remaining constant throughout. A nutrient solution strongly alkaline from the presence of K_3PO_4 became approximately neutral. Hoagland has also emphasized an important point appreciated likewise by some earlier investigators, namely, that the equivalence of positive and negative ions in the solutions is maintained, and the state of equilibrium, the recognition of which is often too vague, is of necessity kept in mind in any discussion of the absorption of ions.

In this paper it has been pointed out that there is generally a decline in growth in solutions A and C when the H-ion concentration is approximately P_H 6. This may be due to the relative insolubility of the phosphates. On the other hand, the generally more favorable growth in the B solution at or approaching neutrality may be related in part to the better distribution of phosphate ions or particles, due to the presence of certain substances in a state of greater dispersity. In this connection it will be recalled that Bonazzi ('19) and Allen ('19) have contributed interesting data on the favorable effects of shaking or agitation, on the growth of *Azotobacter chroococcum*, an organism necessarily grown in alkaline solutions.

Toole and Tottingham's ('18) results, showing increased yield with the addition of ferric hydroxide to Knop's solution, are also of particular interest in considering the data obtained in this work with solution B. At present there seems to be no basis for a final opinion on the rôle of aluminium in promoting growth in these experiments. The high adsorptive property of the compound used, together with its buffer action, may be concerned in the explanation. The maximum effect of this compound occurred with wheat, but in view of the complication of factors involved this may not be significant.

In the various series of experiments here reported there is

considerable diversity in the intervals between the renewal of solutions. These intervals have varied from four days to a time interval covering the entire culture period. The results have been as consistent as might be expected, and it is believed that the value of lengthening the interval in this way is important from the standpoint of reducing the labor in the maintenance of such cultures. Trelease and Free ('17) have, however, shown that frequent changes of solution are more favorable for growth; but it also appears from their data that renewals during the first two weeks are not so important as those made later. They suggest that a continuous flow of solution through the culture is more beneficial than a daily change.

The work of Pantanelli ('15) and others has shown that after plants have been for a few hours in contact with salt solutions it may be demonstrated that there has been a different rate of absorption of the various ions. The solution, therefore, changes rapidly in the presence of abundant absorbing surfaces. Hoagland has also emphasized this point, and within a certain range of osmotic concentration he regards the initial concentration of any particular ion as practically immaterial. Nevertheless, it would be admitted by all that there must be on the one hand a true physiological balance, and that on the other hand, the concentration of no necessary ion or molecule shall become a limiting factor in growth. It is not, however, proposed to discuss in this paper the significance and final results of the interchange of ions or molecules between roots and solutions.

Discussing limiting factors in water cultures Stiles ('16) has drawn attention to the limited application of the water-culture method in physiological problems. He regards this as related to "(1) the difficulty in analyzing results due to the complex of factors not under control; (2) the difficulty of controlling in some cases even the factor whose action is being investigated; and (3) the excess of labor required to produce results which are only of a low degree of accuracy."

In a previous paper Stiles ('15) has in a measure crystallized the feeling of many investigators working with water cultures in arriving at the conclusion that it is necessary to calculate the probable error of the results in accurately evaluating the significance of differences exhibited by different sets of cultures.

In a study of probable error he grew single plants in each of 10 bottles of 1200 cc. capacity, employing uniform methods and seed of a selected strain of rye. Four concentrations of nutrient solutions were used with the different lots. Experiments conducted during the early months of the year yielded results as follows: The greatest individual variation in any one lot amounted to never less than 70 per cent. In the weakest concentration the individual variation was 333 per cent. However, the probable error of the mean in these cases was only about 3–10 per cent of the mean dry weights. Comparable differences were found in cultures made later in the spring with a pure line of barley.

That considerable variability has been found by others is evident from the examination of the tables in any case in which the data have been given in detail. Livingston and Tottingham ('18) give a table from which it appears that while the culture R_1C_3 yields only a fair growth of roots the dry weight for the entire plant is third and for tops is the highest of all in the series. This solution, however, differs from R_8C_1 in containing one-eighth the concentration of KNO_3, 3 times as much $Ca(H_2PO_4)_2$, and 6 times as much $MgSO_4$. R_8C_1 is regarded as "the best balanced for young wheat plants of all the nutrient solutions so far noted in the literature."

The Shive solution, on the one hand, and the Livingston-Tottingham solution, on the other, were of course designed with the idea of simplification. The 3 salts employed contain all the essential ions except iron. Theoretically, the phosphate, nitrate, and sulphate ions may be added in the form of the salts of either of the 3 bases or cations, K, Ca, and Mg. However, the relatively low solubility of Ca as sulphate may seem to render it less practicable to use this salt. There remain 7 possibilities in the selection of salts, and it might appear that in the selection of these the only important points might be, first, H-ion concentration, and second, the use of a base required in relatively low concentration with an anion which may be similarly reduced in strength.

From the emphasis in the literature, as is well known, an important consideration is an appropriate ratio between the ionic proportion of Ca and Mg. Other antagonistic relations

also require consideration. An analysis of the differences between the Shive and the Livingston-Tottingham solution shows that the latter contains a relatively greater concentration of K and NO_3 ions and a lower concentration of Ca, Mg, PO_4, and SO_4. Serious typographical errors in one of their tables (table three, page 345) have led Livingston and Tottingham ('18) into error in the statement that in the R_5C_2 solution there are 2.89 times as many atoms of Ca as of K per unit volume. As a matter of fact the partial volume atomic concentration of the R_8C_1 solution of Livingston and Tottingham contains more than 10 times as much K as Ca, while R_6C_1 contains more than 8 times as much K as Ca, and nearly 6 times as much Mg as Ca. The Mg : Ca ratio of R_8C_1 is very nearly 2 : 1. In both of the best Livingston-Tottingham solutions the ionic concentrations of K and NO_3 are greatest. It seems quite probable that this factor, together with the variability in H-ion concentration of KH_2PO_4, is accountable for the better growth in these solutions.

Summary and Tentative Conclusions

The experiments reported in this paper were undertaken primarily to determine the influence of variations in hydrogen ion concentration on the yield of certain seed plants in solution cultures. As the work progressed, however, many modifications were suggested, and some of these involved in no way a consideration of hydrogen ion concentration at points which might be regarded as critical for the growth of the crops used.

The selection of several culture solutions seemed necessary in order that some diversity might be introduced in the salt proportion or composition factors. The solutions employed, and their designations, were as follows: solution A, a slight modification of one of Shive's "best" solutions; solution C, a slight modification of one of the best Livingston-Tottingham combinations; and solution B, based in part upon the Crone combination of salts, but with this essential difference, namely, that "soluble ferric phosphate" was used in place of the "insoluble" iron salt. Each of the solutions first mentioned contains a monobasic phosphate, and with theoretically pure chemicals should yield culture solutions with a P_H exponent about 4.5. Solution B may vary, in my experience, from a hydrogen ion

concentration represented by P_H 5.4 to P_H 7.1; frequently, however, it is 6.6 to 7.1.

The experiments have been carried out in the greenhouse at different periods of the year, and represent, on the whole, a considerable range of environmental conditions. It has been impracticable to analyze these except in a very general way, or relatively. Wheat and corn have been employed in every series of experiments and Canada field peas in all series here reported except one.

Under the most favorable conditions, the 3 solutions mentioned above, without other modification, may all yield excellent growth. Plants grown in solution B are invariably of a deeper green, presenting a finer appearance, and the average of the growth quantities (green weight) is higher for wheat and corn than in either of the other 2 solutions. In the unmodified solutions A and C the green weight of peas averages higher than in the unmodified solution B.

Culture solutions prepared with monobasic phosphates may, however, exhibit a hydrogen ion concentration which is too high for the maintenance of the best growth under certain conditions, and especially is this true in the case of wheat.

Solutions made with monobasic potassium or calcium phosphate free from acid may, under certain conditions, yield maximum growth quantities, but there is often considerable variability in the duplicate cultures due to unknown factors. Certain grades of the phosphates mentioned—if not specially purified in the laboratory—exhibit a P_H which may be distinctly toxic. Correction of the P_H to about 4.8 or 5.2 by means of NaOH or by the use, in part, of a dibasic salt generally affords increased growth.

Under extreme conditions—effecting a high evaporation rate—it becomes more important to correct to the higher P_H exponent. Wheat, corn, and peas are sensitive in the order named to high hydrogen ion concentration.

Usually, the addition to solution B of small amounts of dibasic potassium phosphate, of solid calcium carbonate, and of aluminium hydroxide has given increased yields, often considerably above that of the unmodified solution. The results in the case of the aluminium compound are notable in the case of wheat

grown under the conditions described,—related in part, presumably, to adsorption and buffer action.

In general, it would seem that there may be no single "best" solution for the growth of any of the 3 plants employed in this work. In all probability a "best" solution, like the "optimum" temperature, is represented within the "optimum" concentration rather by considerable range of salt or ion proportions, influenced to a greater or less degree by environmental factors.

If the initial P_H of the culture solution is considerably less than neutrality there is generally a tendency for this to be shifted toward the neutral point, although this depends in part upon the composition of the solution and in part upon the plant grown.

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HUMIDITY IN RELATION TO MOISTURE IMBIBITION BY WOOD AND TO SPORE GERMINATION ON WOOD

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INTRODUCTION

In a previous publication the writer ('16) pointed out that between a certain minimum and maximum of moisture in wood *Lenzites saepiaria* and other similar wood-destroying fungi will grow and cause the destruction of the wood. Thus, the power of wood to absorb moisture, whether as vapor from the air or as water from objects in contact with the wood, is a factor of prime importance in its susceptibility to decay. It is no less true that any property of the wood which may influence its moisture-absorbing capacity is a factor in its durability. In this connection it has been suggested (Zeller, '16) that, although resin has no actual toxic effect on the growth of wood-destroying fungi, it does inhibit growth when in large percentages and its only inhibitive power probably lies in the fact that it excludes water from the fibre containing it (Zeller, '17).

With these previous results as a foundation further investigations have been conducted to ascertain (1) the amount of moisture which wood will absorb from the atmosphere at different relative humidities when the temperature remains constant; (2) whether the water-proofing effect of resin on wood can be measured; and (3) the relation of the moisture content of wood (or relative humidity of the atmosphere) to the propagation of wood-destroying fungi on wood. The purpose of the present paper is to report the results of these experiments.

EXPERIMENTATION

Materials used.—For the experiments reported below samples were selected from specimens of shortleaf pine (*Pinus echinata*) secured at the Fordyce Lumber Company, Fordyce, Arkansas, and from specimens of longleaf pine (*Pinus palustris*) secured at the

Calcasieu Lumber Company, Lake Charles, Louisiana. From these samples approximately 250 specimens of each species were selected, so that a wide range in resin content and specific gravity as well as sap- and heart-wood might be represented.

The samples were allowed to air-dry for several weeks in a room at about 50 per cent relative humidity. They were then measured for volume and weighed, and the specific gravity determined for the whole sample. The samples were usually 2×6×48 inches. Of course, these specific gravities are not standard (Newlin and Wilson, '19), for the specimens were not *oven-dry* when weighed. Since the samples were obtained for the purpose of measuring the moisture-absorbing power of the wood they were not kiln-dried, for this tends to reduce the hygroscopic property of the wood below that obtaining in green samples (Tiemann, '07). This point was kindly called to my attention by C. H. Teesdale of the Forest Products Laboratory, Madison, Wisconsin, and in a report from that laboratory on "Wood in aircraft construction" (1919) the relation of kiln-drying of wood to its hygroscopicity is quite generally discussed. All samples finally selected for the moisture-absorption tests were marked and so labeled that after they were cut into blocks 2×2×4 inches, each block could be identified as to which sample it belonged and as to its position in the sample. From these small blocks uniformly clear pieces were selected for the preparation of shavings which were uniform in thickness. A preliminary experiment revealed the fact that the moisture-imbibing power of wood is not changed by shaving, but that imbibition is more rapid than when blocks were used. Therefore, in the experiments reported below shavings were used. The clear pieces selected were of a light color and had a resin content well below 5 per cent, except those otherwise designated in tables II and IV.

Description of humidors.—In order to determine the relation between the humidity gradient of the atmosphere and the moisture content of wood a closed chamber, or humidor, was devised so that a constant temperature would be maintained, and so that the humidity could be regulated either by different concentrations of sulphuric acid in trays enclosed in the chamber, by varying the evaporating surface of water in trays, or by hanging baskets of calcium chloride in the chamber. To pro-

duce the higher humidities, curtains of absorbent paper were hung in the chamber so that the lower portions were in contact with water in trays. This increase in evaporating surface proved very effective.

The humidor with apparatus complete as we used it is shown in plate 1. The humidor proper consists of two double-walled boxes, one within the other, built of spruce lumber. The double walls are packed with sawdust for insulation against temperature changes. For added insulation there is an air space separating the inner and outer box. The inner box, which is the chamber proper, has a double-walled door provided with a double-glass window, through which temperature and dew-point observations can be made without opening. The outer door is double-walled and packed with sawdust. The doors are provided with ordinary cold-storage catches. There are three one-inch openings, one through the top and one through each end. The inner walls of the humidor chambers were water-proofed in two ways: (1) Those of two humidors were brushed with hot paraffin (parawax) and were then thoroughly ironed with a hot electric iron. Further applications of the paraffin were made in the same manner. (2) The walls of two other humidors were primed and painted with several coats of a water-proof enamel and valspar. The paraffin treatment does not give as good an appearance as the enamel paints, but it proved to be the better water-proofing agent.

The temperature of the chamber was maintained at 25° C. by means of a bimetallic thermo-regulator in circuit with an electric light as a heating element.

Determination of relative humidity.—There was no difficulty in maintaining a constant relative humidity throughout any one experiment. The relative humidity of the chamber was determined by means of a Milliken dew-point apparatus, which consists of a highly polished, nickel, cylindrical cup provided with a three-hole stopper. One of these holes supports a thermometer and the other two provide an intake and outlet for the siphoning of water or freezing mixtures through the cup. This apparatus shows plainly in fig. 2, pl. 1. The dew-point is determined by the appearance and disappearance of the film of moisture on the polished cup as the temperature is changed by

allowing water to flow through the siphon. From the vapor pressures of the dew-point and the temperature of the chamber the relative humidity is obtained. The pressure of aqueous vapor at various temperatures was secured by recourse to the Smithsonian physical tables (Fowle, '10).

Method of weighing within the humidor.—The samples of wood shavings which were used to measure the imbibition of moisture from the atmosphere were planed as needed and placed in the wire baskets shown in fig. 1 and 2 of pl. 1. The baskets provided with hooks were hung on two wires which were stretched across the chamber, one on either side of the openings in the top and the right-hand side of the chamber. Balances were installed on the top of the humidor in such a position that a wire supporting a counterweight passed down through the opening leading to the chamber. The lower end of this wire is hooked so that the wire baskets can be hung upon the balance by means of a lever which passed into the opening in the right-hand end of the humidor. This operation can be performed without opening the chamber and thus changing the temperature and humidity within. The wire lever and the counterbalance wire are each provided with a cork, which can be slipped back when the device is in use or into the apertures when not in use. This device is an adaptation of that described by Dixon ('98).

A similar one to that described by the writer has been used for the same purpose by Mr. C. H. Teesdale in charge of the Section of Wood Preservation at the Forest Products Laboratory, Madison.

Determination of moisture content of wood.—After the samples of shavings had come to a constant weight in the humidor they were weighed and at the same time the relative humidity of the chamber was determined and recorded. The samples were then dried to constant weight in a dry-air oven at 100–105° C. The difference between the weight taken in the chamber and that after drying gave the total amount of moisture in the samples including that absorbed at the relative humidity of the chamber. The per cent of moisture absorbed was based on the oven-dry weight of the shavings.

Determination of resin content of wood.—To determine whether the influence of a high resin content on moisture absorption can

be measured experimentally samples were chosen and analyzed by the method previously reported (Zeller, '17).

Results of Experiments on Moisture Absorption

The data from the experiments to show the relation between the relative humidity of the atmosphere and the moisture content both of sap- and heart-wood of shortleaf and longleaf pine are given in tables I, II, III, and IV.

From the data recorded in these four tables (I, II, III, and IV), the humidity-moisture curves shown in figs. 1, 2, 3, and 4, respectively, were plotted. These curves represent the ultimate moisture content at a given temperature and relative humidity of the surrounding atmosphere. The samples used in each experiment were of three distinct specific gravities chosen to represent the approximate average specific gravity of the species of wood, as well as densities both heavier and lighter than the average. In the case of heart-wood, however, an extra series of samples was used. These were highly resinous and consequently very dense. Other than these highly resinous pieces, the samples had a resin content well below 5 per cent.

The four curves are strikingly similar. In all cases where the samples of wood represented have specific gravities lying between .41 and .75 to .80 and small percentages of resin, the general curve for the moisture absorbed is followed up to a certain relative humidity where the percentage of moisture taken up by the wood of the three densities begins to vary according to the density. The lighter, or less dense, samples, from this point, take up more moisture with increased atmospheric humidity than do the denser samples. This occurs at a relative humidity averaging from 94.75 to 96 per cent. There seems to be but one explanation for a divergence of the curves at this juncture. Up to this point the wood fibre has not received enough moisture from the atmospheric humidity to satisfy its imbibition capacity, but beyond this point this hydration capacity is over-satisfied and the moisture over and above that absorbed by the fibre is adsorbed by the surfaces exposed. If this theory is correct the point of divergence of the three moisture-humidity curves represents the fibre-saturation point. As an irreversible colloid, wood is undoubtedly limited in further

TABLE I

PERCENTAGE MOISTURE CONTENT OF SHORTLEAF PINE SAP-WOOD SHAVINGS AT VARIOUS ATMOSPHERIC HUMIDITIES AND AT 25° C.

Samples of .41 specific gravity		Samples of .61 specific gravity		Samples of .69 specific gravity	
I	II	III	IV	V	VI
Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Moisture in shavings (per cent)	Relative humidity of chamber (per cent)
4.12	11.0	2.5	6.6	3.0	7.5
5.50	19.5	6.62	24.8	4.87	15.25
7.25	28.0	7.5	31.8	6.12	22.75
7.80	33.2	7.9	35.8	7.88	34.6
8.70	45.5	8.4	40.4	8.70	42.5
9.00	49.5	9.2	47.5	9.7	53.
9.88	53.8	9.5	54.5	10.75	62.5
10.62	58.8	10.3	58.8	11.6	66.0
10.80	60.4	10.4	61.3	12.6	69.5
11.12	63.8	11.5	65.0	14.25	76.8
12.80	68.8	12.2	67.5	15.3	79.3
13.88	73.8	13.0	71.8	16.3	84.4
15.5	81.0	16.2	82.2	19.3	91.0
17.25	86.5	17.6	86.4	23.4	95.3
18.5	87.8	20.0	90.0	24.5	96.5
18.75	90.0	21.6	93.2	25.0	97.5
21.4	92.25	24.25	96.0	26.0	98.2
22.3	94.4	26.0	97.4	26.25	98.8
23.8	95.5	26.6	98.2	27.1	99.3
25.4	96.6	27.12	98.2	27.75	99.5
27.6	97.5	27.6	98.7	28.0	100.0
28.9	98.4	28.6	99.2		
30.25	98.7	29.0	99.5		
31.5	99.2	29.88	100.0		
32.0	99.5				
34.0	100.0				

TABLE II

PERCENTAGE MOISTURE CONTENT OF SHORTLEAF PINE HEART-WOOD SHAVINGS AT VARIOUS ATMOSPHERIC HUMIDITIES AND AT 25° C.

Samples of .49 specific gravity		Samples of .61 specific gravity		Samples of .70 specific gravity		Resinous samples of .82–.90 specific gravity		
I	II	III	IV	V	VI	VII	VIII	IX
Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Per cent resin
3.8	10.3	3.2	8.0	3.875	9.4	6.62	24.8	15.6
4.75	13.6	6.2	20.4	5.12	15.6	7.9	33.6	16.2
6.5	23.2	7.0	26.2	6.00	19.0	8.5	38.5	16.7
7.2	30.2	7.6	31.8	7.12	27.8	9.32	43.5	16.2
7.5	33.2	7.7	34.6	7.5	30.3	10.2	49.6	15.6
9.0	41.5	8.4	36.	8.4	37.2	10.34	54.4	18.2
10.7	51.8	8.7	40.4	9.8	45.6	11.32	57.4	17.6
12.3	61.0	10.1	44.2	11.38	55.8	12.3	63.0	17.6
14.5	71.0	10.2	47.4	13.18	65.8	13.7	70.0	16.7
16.7	78.0	11.2	53.8	15.86	75.6	13.88	72.6	18.2
18.0	82.4	11.8	59.3	16.4	79.2	14.8	75.6	15.6
19.7	87.5	13.12	63.2	17.37	80.4	16.0	80.4	18.2
21.75	92.0	12.75	65.0	18.62	86.0	16.28	83.2	15.6
23.3	94.5	13.6	67.5	20.7	90.0	17.5	86.0	16.2
24.0	95.0	15.6	72.8	22.12	93.0	18.25	88.2	16.2
25.5	96.0	16.14	76.6	22.75	94.0	18.7	90.6	15.6
28.8	97.8	17.3	78.8	24.25	95.60	19.5	91.4	18.2
30.7	98.4	18.4	84.4	25.2	97.0	20.11	94.4	17.6
32.55	98.8	19.4	85.3	28.08	100.0	20.52	96.2	18.2
36.25	100.0	19.7	88.6			21.4	96.6	18.2
		20.3	88.8			21.41	98.6	17.6

TABLE II—*Continued*

PERCENTAGE MOISTURE CONTENT OF SHORTLEAF PINE HEART-WOOD SHAVINGS AT VARIOUS ATMOSPHERIC HUMIDITIES AND AT 25° C.

Samples of .49 specific gravity		Samples of .61 specific gravity		Samples of .70 specific gravity		Resinous samples of .82–.90 specific gravity		
I	II	III	IV	V	VI	VII	VIII	IX
Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Per cent resin
		21.2	91.			22.25	100.0	17.6
		22.6	93.2					
		24.75	96.2					
		25.75	97.0					
		27.12	98.0					
		29.32	98.8					
		31.25	99.4					
		33.10	100.0					

TABLE III

PERCENTAGE MOISTURE CONTENT OF LONGLEAF PINE SAP-WOOD SHAVINGS AT VARIOUS ATMOSPHERIC HUMIDITIES AND AT 25° C.

Samples of .41–.42 specific gravity		Samples of .70 specific gravity		Samples of .75–.80 specific gravity	
I	II	III	IV	V	VI
Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Moisture in shavings (per cent)	Relative humidity of chamber (per cent)
3.88	9.3	4.64	12.3	5.4	15.8
6.78	28.10	6.24	21.4	6.89	26.1
8.24	37.4	7.21	28.2	7.88	34.0
9.52	47.8	7.30	31.4	8.64	40.6
10.83	56.4	9.21	44.7	9.78	50.8
12.2	64.2	10.11	49.4	10.68	55.1
13.77	71.6	10.38	53.6	11.27	61.2
14.82	75.8	11.50	58.6	12.77	67.3
17.59	83.7	12.54	65.8	15.73	79.0
20.31	88.2	13.42	69.3	19.32	87.2
22.38	92.5	14.15	73.8	21.71	91.2
24.14	94.6	16.51	80.3	23.58	94.6
24.88	95.4	17.07	82.5	23.96	95.4
25.76	96.2	18.48	85.5	24.54	96.1
26.63	96.7	20.74	89.7	24.74	97.2
28.64	97.8	23.35	94.2	25.32	97.7
30.52	98.7	23.76	94.6	26.14	98.8
32.18	99.1	24.26	95.3	27.02	100.0
35.76	100.0	25.61	96.8		
		26.80	97.4		
		27.63	98.6		
		28.59	99.1		
		30.28	100.0		

TABLE IV

PERCENTAGE MOISTURE CONTENT OF LONGLEAF PINE HEART-WOOD SHAVINGS AT VARIOUS ATMOSPHERIC HUMIDITIES AND AT 25° C.

Samples of .51 specific gravity		Samples of .70 specific gravity		Samples of .75–.80 specific gravity		Resinous samples of .86–.94 specific gravity		
I	II	III	IV	V	VI	VII	VIII	IX
Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Moisture in shavings (per cent)	Relative humidity of chamber (per cent)	Per cent resin
3.89	10.6	3.68	9.8	4.12	11.8	6.25	23.1	19.3
4.48	12.6	5.0	15.0	5.62	18.6	7.24	31.2	17.1
5.32	17.6	5.88	20.0	7.14	26.6	7.9	38.3	18.4
6.78	26.0	7.14	28.2	7.16	29.4	9.42	51.6	16.4
7.82	31.6	7.64	33.0	7.8	34.8	9.36	55.4	19.3
8.84	46.6	8.25	40.0	8.88	49.6	9.71	58.7	19.3
10.08	54.8	8.62	42.2	9.87	53.0	10.58	58.7	18.4
11.06	59.8	9.28	48.2	10.21	57.6	10.27	60.8	17.1
12.2	66.4	9.31	50.0	10.68	61.2	11.28	64.4	16.4
13.8	74.4	10.78	60.0	11.28	62.5	11.76	66.0	16.4
15.52	81.4	11.64	63.6	11.72	65.1	12.26	68.7	18.4
17.10	85.4	12.72	70.0	13.38	72.9	12.2	70.8	16.4
20.10	90.8	16.22	83.8	14.27	76.6	12.81	71.7	17.1
21.78	93.4	17.4	86.8	18.54	88.3	13.02	74.3	18.9
23.68	95.6	19.21	89.4	19.71	90.7	13.94	78.0	17.1
25.1	96.6	22.79	94.6	20.86	92.1	14.31	78.7	16.4
26.62	97.4	24.36	96.51	23.27	95.6	14.82	82.4	19.3
27.8	98.0	26.11	98.	24.01	96.5	15.13	84.7	19.3
30.32	99.0	27.57	99.2	25.24	97.9	16.08	86.9	17.1
33.2	100.0	29.1	100.0	26.11	99.1	17.0	90.3	19.0
				26.89	100.0	16.75	91.5	16.4
						18.48	93.5	19.0
						17.41	93.8	19.3
						18.74	95.1	19.0
						19.66	95.3	16.4
						20.14	97.2	18.4
						20.15	99.1	18.4
						21.12	100.0	18.4

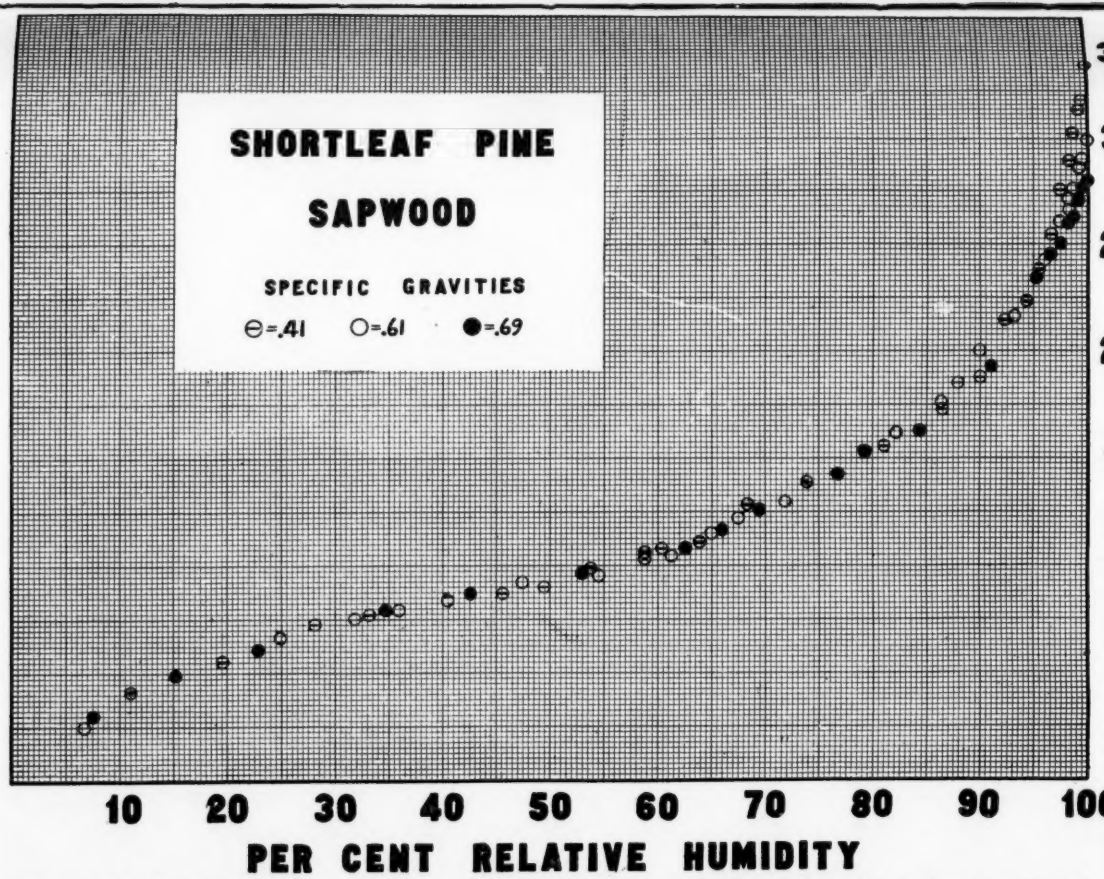

Fig. 1. Curve showing the moisture content of the sap-wood of shortleaf pine (*Pinus echinata*) at various atmospheric humidities and at 25° C.

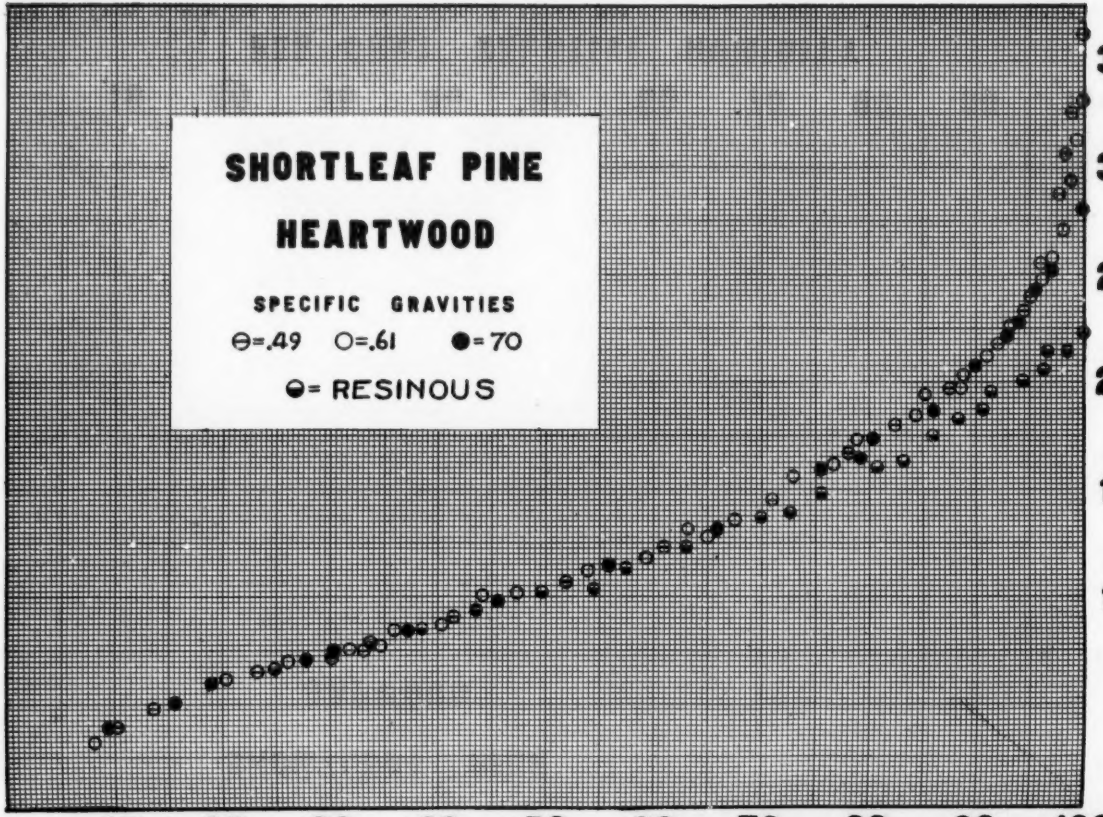

Fig. 2. Curve showing the moisture content of the heart-wood of shortleaf pine (*Pinus echinata*) at various atmospheric humidities

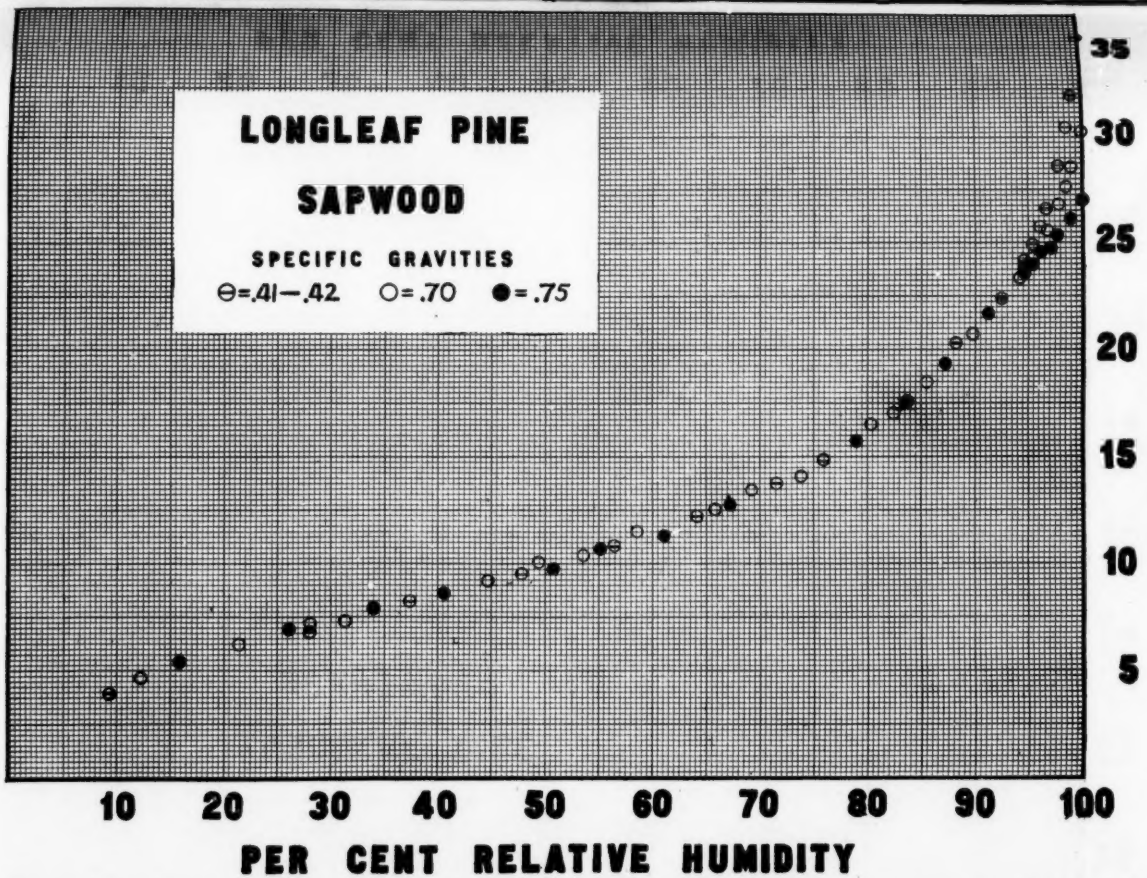

Fig. 3. Curve showing the moisture content of the sap-wood of longleaf pine (*Pinus palustris*) at various atmospheric humidities and at 25° C.

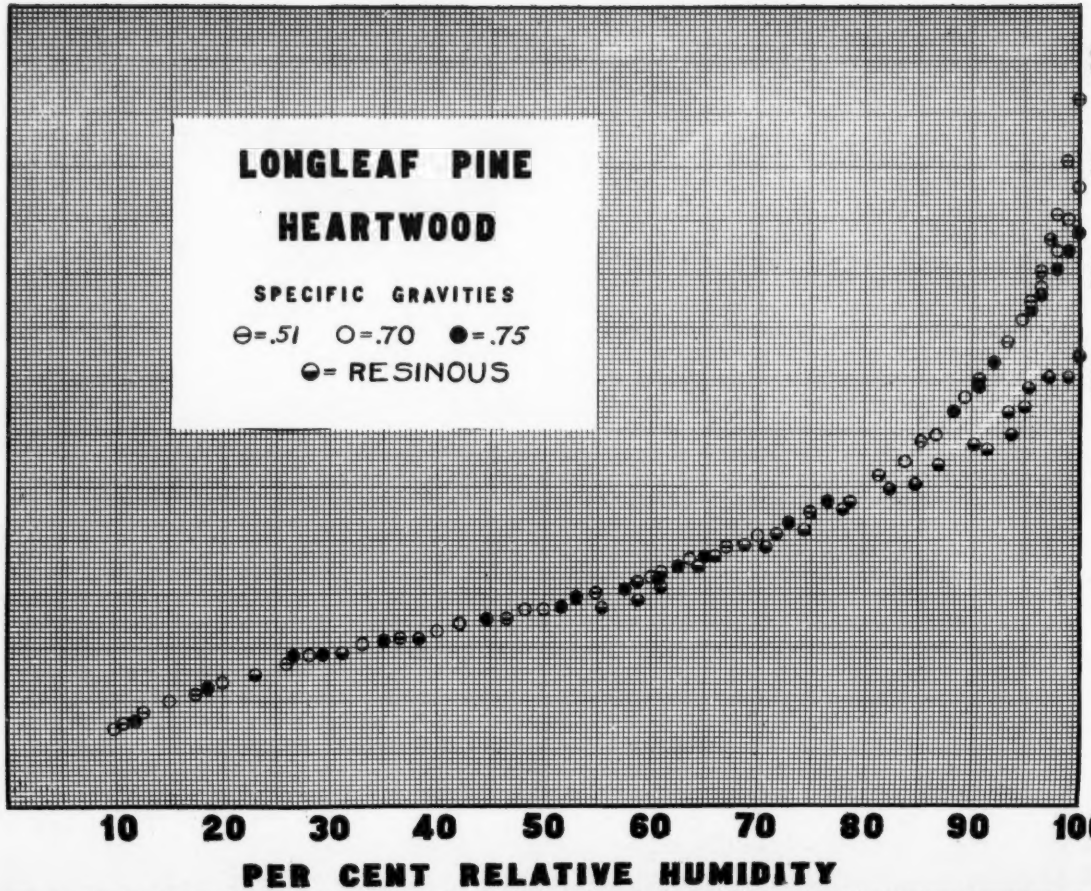

Fig. 4. Curve showing the moisture content of the heart-wood of longleaf pine (*Pinus palustris*) at various atmospheric humidities and at 25° C.

absorption of moisture by certain morphological and mechanical properties of the woody tissues which vary with the different species of wood. This variation in morphological and mechanical characters in the wood of different species will account for the slight differences in moisture content at the fibre-saturation point of shortleaf and longleaf specimens. The moisture content at the so-called fibre saturation point of the four curves is approximately as follows: shortleaf pine sap-wood, 24.25 per cent, heart-wood, 24.5 per cent, and longleaf pine sap-wood, 23.75 per cent, and the heart-wood, 23.25 per cent.

In timber-testing laboratories the fibre-saturation point of wood has been determined by means of strength tests, and has been defined by Tiemann ('07) as "the degree of moisture at which maximum absorption by the cell walls is reached." After this point is reached added moisture does not lessen the strength. Beginning with the dry conditions, with the increase of moisture the strength falls off very rapidly at first, then more slowly as the fibre-saturation point is reached, and here it abruptly ceases to decrease. This abrupt break in the moisture-strength relation represents the fibre-saturation point. The moisture per cent at fibre-saturation for longleaf pine as determined by Tiemann ('07) by compression tests on small specimens averaged 25 per cent, with a maximum at 26 per cent and a minimum at 24 per cent. This is not far removed from the moisture content at which the curves representing the three different specific gravities of longleaf pine diverge in figs. 1 and 2.

If this divergence of the moisture-humidity curve represents the fibre-saturation point the appreciable increase in moisture up to 100 per cent humidity must be moisture in the form of a surface film; that is, the fibre-saturation point is the point where absorption or imbibition by the fibre is replaced by a surface phenomenon, adsorption. In a unit weight of wood fibre the thin-walled, large-lumened cells, having a lower specific gravity than the heavy-walled, small-lumened cells, present a much greater surface than the latter. If the greater concave curvature of the smaller-lumened cells has any tendency to thicken the surface film in proportion to that adsorbed by those having less curvature, the difference in the total moisture created in this way evidently is not great enough to overcome the difference resulting from the difference in surface.

PER CENT RELATIVE HUMIDITY

Fig. 4. Curve showing the moisture content of the heart-wood of longleaf pine (*Pinus palustris*) at various atmospheric humidities and at 25° C.

This relation of the moisture content of wood at various relative humidities has been demonstrated for other woods by workers at the United States Forest Products Laboratory located at Madison, Wisconsin. This work is reported in "Wood in Aircraft Construction" (1919) which was prepared by the above-mentioned laboratory for the United States Navy Department. The five woods worked with were Sitka spruce, black walnut, white oak, yellow birch, and ash, and the maximum moisture content at 100 per cent humidity ranged from about 29.5 to 30.2 per cent for Sitka spruce to about 36.7 per cent for ash. A report by Pfeiffer on the physical properties of several Javanese woods includes a study of their moisture contents at various atmospheric humidities. The maximum moisture contents of the various woods at 100 per cent humidity were as follows: *Tectona grandis* (teak wood), 21 per cent; *Ensideroxylon Zwageri*, 22 per cent; *Shorea* sp., 24 per cent; *Dipterocarpus* sp., 23 per cent; *Shorea* sp., 30 per cent, and *Alstonia* sp., 25 per cent. The specific gravities of these woods were .64, 1.04, .88, .64, .41, and .34, respectively. Although the order of increase in maximum moisture content in these Javanese woods is not in exactly the same order as the decrease in their specific gravities, there is a tendency in that direction. Of course, a direct relation throughout could not be expected because of the great differences in morphological and physical structures between various species.

Resin Content in Relation to Moisture Absorption

The data on this subject as presented in tables II and IV and illustrated graphically in the curves shown in figs. 2 and 4 are self-explanatory and for the most part need no discussion. However, there are a few points of interest which might be discussed briefly. In the lower part of the curves, where the relative humidity is less than 50 per cent, the points representing the highly resinous samples of wood show no deviation from the moisture curves of those samples of wood containing less than 5 per cent resin. Above 50 per cent humidity, however, there is a gradual deviation of the curve representing highly resinous specimens. This moisture curve is lower than that representing specimens containing less than 5 per cent resin. This illustrates the fact that resin actually has a water-proof-

ing effect upon the wood containing it, and that this water-proofing effect is considerable as the fibre-saturation point is approached. In the shortleaf heart-wood at a relative humidity of 95 per cent the moisture absorption was decreased 15.6 per cent by 17.6 per cent resin, and at 100 per cent humidity the minimum moisture decrease was 20.8 per cent, and the average decrease was 31.5 per cent brought about by 17.6 per cent resin. In the longleaf pine heart-wood at a relative humidity of 95 per cent a resin content of 16.4–19 per cent had a water-proofing effect of 17.4 per cent, while at 100 per cent humidity the water-proofing effect of 18.4 per cent resin averaged 29.3 per cent, with a minimum of 21.5 per cent reduction in the moisture content.

Undoubtedly, this water-proofing effect of resin has its influence upon the durability of structural timber placed under *very* humid conditions, providing the resin content is sufficient to lower the moisture content of the wood below that which is conducive to the growth of wood-decaying fungi. Although the resin does have some influence in this direction, it is probably not sufficient to be relied upon as a test of durability. More reliance could be put upon resin as an index of durability in timbers containing, for example, 12 to 15 per cent resin, providing there was any reason to believe that it was equally distributed throughout the timber. With such an equal distribution of the resin the water-proofing would undoubtedly be more effective at lower relative humidities. Not only this, but mechanical resistance of resin equally distributed would undoubtedly be a great factor in the inhibition of fungous growth in the wood. This, however, is not the case. The resin is deposited in streaks in wood so that there are portions relatively free from resin. These resin-free portions, having a hygroscopicity sufficient to take up considerable moisture under the right moisture conditions, become sources of weakness because of the inroads of wood-destroying fungi. Examples of such decay are often reported as very destructive to the ceilings and structural timbers under the highly humid conditions produced in the paper- and pulp-mills of the eastern states and Canada and the knitting-sheds of the cotton industries of New England. Further experimentation along this line is advisable. Some practical process of treating resinous lumber, possibly by modifying the kiln-drying

processes so as to distribute the resin more evenly, is a worthy project of investigation of this important problem in these industries.

SPORE GERMINATION ON WOOD

The spores of wood-destroying fungi exhibit a marked similarity with regard to their requirements for germination. Some of the factors which influence germination are proper temperature and proper amounts of acidity and moisture. As early as 1860 Hoffmann ('60) found that in a period of five days spores of *Polyporus versicolor* germinated in moist air, and in a period of six days spores of *P. squamosus* germinated better in moist air than in water. On the other hand, Falck ('09) says that the spores of *Lenzites* on wood germinate only when the wood has been thoroughly saturated by rains.

To determine more accurately the relation of moisture to the germination of spores of a wood-destroying fungus the following experiments were conducted, using the spores of *Lenzites saepiaria*. The spores of this fungus were obtained from rejuvenated fruiting bodies as described in a previous paper (Zeller, '16). The spores were allowed to drop from the fruiting bodies directly upon thin shavings of shortleaf pine (*Pinus echinata*) sap-wood, or were caught in Petri dishes and transferred to the shavings in a loop of sterile distilled water and allowed to dry rapidly in the air.[1]

The shavings upon which the spores were deposited were clamped in a small device which may be described as follows: Two pieces of sheet celluloid, about the size of a microscope slide, were riveted together at one end by means of a paper rivet, and a round hole about 1 cm. in diameter was cut through the middle of the two celluloid slides. The pieces of celluloid were bent apart while a shaving to which the spores were adhering was slipped between, the other end of the pieces of celluloid being clamped together by means of a paper clip. The shaving is thus held so that it covers the hole in the middle of the devic e

[1] A decoction from 60 gms. of shavings of shortleaf-pine sap-wood was prepared by steaming the shavings in a reflux with 10 cc. of distilled water. This decoction tested P_H 4.2, so that the actual active acidity of the shavings probably was not above P_H 3.8. This would be well toward the optimum acidity (P_H 3.1) for the germination of the spores of *Lenzites saepiaria* as reported by Webb ('19).

and is exposed to the air from both sides. These devices were numbered, and after the chamber of the humidor, described elsewhere in this paper, had come to a constant humidity, one of them was lowered into the chamber through the opening in the top. By means of the lever it was hung on a wire without opening the chamber. In this way spores were exposed to the atmospheric conditions of the chamber without coming in contact with any other object. A new shaving was added to the chamber on 12 successive days. All were removed at one time, examined under the microscope, and the percentage of germination estimated on the basis of the total number of spores in a unit field. In table v the percentages of germination

TABLE V

RELATION OF THE GERMINATION OF THE SPORES OF LENZITES SAEPIARIA TO THE RELATIVE HUMIDITY OF THE ATMOSPHERE AT 25° C.

Germination period (days)	Per cent germination (averages)	Per cent relative humidity	Germination period (days)	Per cent germination (averages)	Per cent relative humidity
10	1.5	63.0	5	76.0	96.5
10	2.5	65.0	5	85.5	98.5
10	3.5	67.4	5	100.0	99.0
10	2.5	72.5	5	100.0	98.0
7	4.0	78.5	5	95.0	98.0
7	7.5	82.5	6	57.5	96.0
7	5.5	85.5	6	80.0	98.0
7	10.0	89.8	6	87.5	98.0
5	20.5	90.5	6	92.0	99.0
5	25.5	92.5	6	96.5	99.0
5	70.0	95.5	6	100.0	98.5

are the averages of three or four counts. In fig. 5 the relation of spore germination to relative humidity is presented graphically. This curve indicates that until the fibre-saturation point is reached the percentage of spore germination is very low, but as soon as the humidity of the air is sufficiently high to supply free

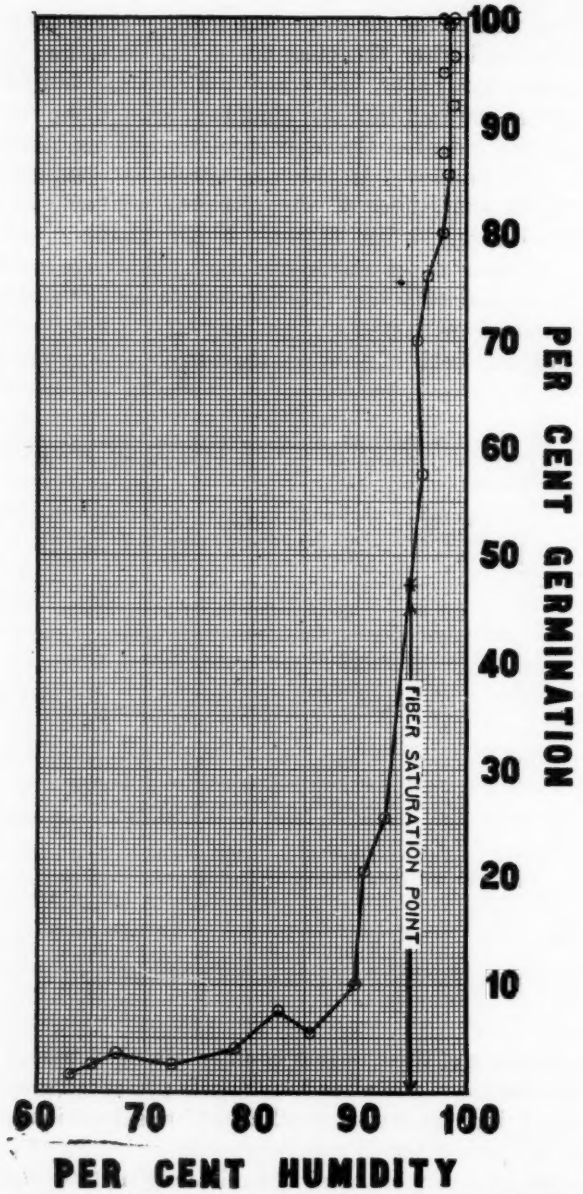

Fig. 5. Curve showing the percentage of germination of spores of *Lenzites saepiaria* on shavings of the sap-wood of *Pinus echinata* at various atmospheric humidities and at 25° C.

water as a film on the wood surface the spores of *Lenzites saepiaria* show a high percentage of germination.

By growing *Ceratostomella coerulea* upon blocks of pine sapwood Münch ('09) found that free-water on the wood was necessary to sustain growth. Growth is maintained if fibre-saturation is maintained, but if the water of imbibition falls below the saturation point the wood seems to demand water at the expense of the mycelium. Although true for *Ceratostomella* this very probably is not true for the mycelium of such fungi as *Lenzites saepiaria*, *Merulius lacrymans*, or *Coniophora cerebella*, which after becoming well established, seem to maintain a water supply from some unknown source. This is especially the case with *Merulius lacrymans*. Wehmer ('14) found that this fungus would not grow on blocks of wood in ordinary cellar humidity unless the blocks were first saturated with water. After the growth was well established, however, the water content of the wood was maintained by the fungus above that in sound wood under the same conditions.

It would seem then that for the germination of the spores and the establishment of the mycelium of wood-destroying fungi, the wood must contain enough moisture to saturate the wood fibre. This becomes a serious problem then in such buildings as paper- and pulp-mills and knitting factories where high humidities are maintained. If the temperatures fluctuate across the dew-point, the moisture content of any exposed woodwork is maintained up to fibre-saturation and the wood is sure to decay unless some method of treatment of the timbers is possible.

Summary

In this paper are reported the results of experiments (1) showing the moisture content of wood at various atmospheric humidities and at 25° C. Curves are presented to illustrate this relation for the sap- and heart-wood of both longleaf and shortleaf pine.

(2) By testing the moisture content of any one species of wood samples having various specific gravities at the various humidities it is possible to approximate the fibre-saturation point of the wood.

(3) The moisture-humidity curves of highly resinous samples

of wood illustrate the water-proofing effect of resin on the wood, especially above 50 per cent humidity. It is believed that resin cannot be relied upon as an indication of the durability of lumber although present in amounts as high as 12–15 per cent, for it is seldom equally distributed in the wood. The regions of low resin content are sources of weakness if wood-destroying fungi gain access to them under favorable moisture conditions.

(4) The germination of the spores of *Lenzites saepiaria* on wood shavings was accomplished at various relative humidities ranging from 63 to 100 per cent. The germination curve illustrates the fact that spore germination is exceedingly accelerated when the atmospheric humidity is high enough to maintain fibre saturation of the wood.

(5) A humidor for maintaining constant humidity and temperature is described. It is provided with (1) a dew-point apparatus for the determination of humidity, and (2) a weighing device sc that the samples can be weighed without opening the humidity chamber.

The writer wishes to express his appreciation of the financial aid accorded him by the Southern Pine Association, without which this work would have been impossible. Thanks are also due the staff of the Missouri Botanical Garden for coöperation and for facilities for the work, and to Dr. Hermann von Schrenk for helpful suggestions.

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Description of Plate

PLATE 1

Fig. 1. A general view of the humidor as described on page 53, with the balances in place and both doors open.

Fig. 2. The interior of the humidor showing the baskets containing the samples of shavings, one basket hanging on the balance and the Milliken dew-point apparatus in the central foreground.

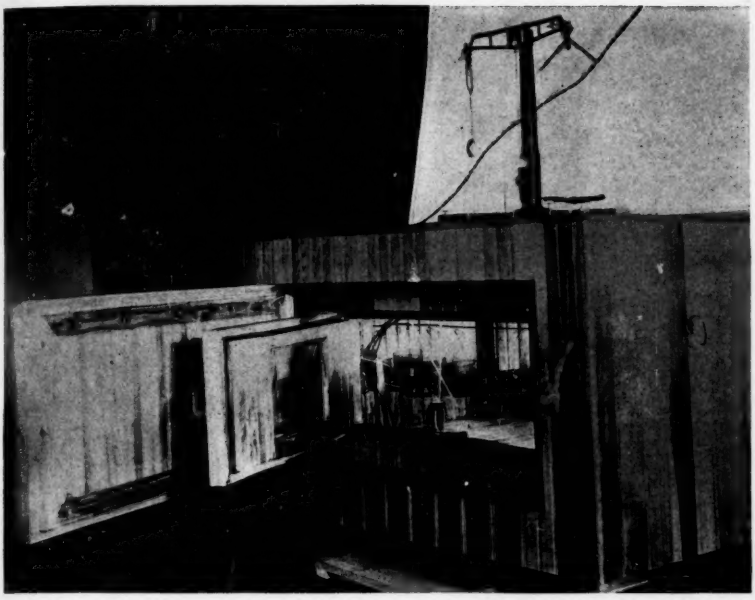

FIG. 1

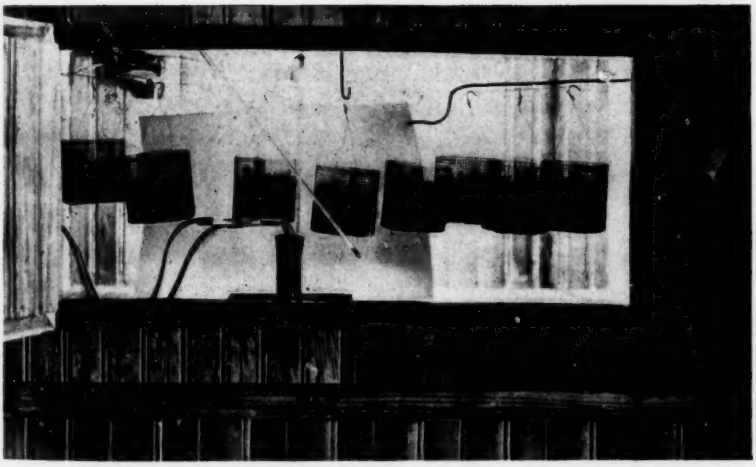

FIG. 2

ZELLER, IMBIBITION BY WOOD, AND SPORE GERMINATION

ON CARBOHYDRATE CONSUMPTION BY AZOTOBACTER CHROOCOCCUM

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In a previous paper (Allen, '19) devoted mainly to the study of the cause of beneficial action resulting from mechanical agitation of *Azotobacter* solution cultures, the defects of existing experimental methods for the study of the physiology of this organism were pointed out. The present report covers a continuance of these studies aimed at the following improvements: (1) renewal of the energy source in the cultures; (2) simultaneous determinations of nitrogen and of residual carbohydrate at short intervals; and (3) mechanical improvements.

The object of the first of these improvements was, of course, to produce heavier growths in the cultures and therefore more marked changes in the amounts of metabolic products, thus increasing the reliability of the measurements of such changes. In view of the theoretical considerations of Duclaux ('98–'00) in regard to the rate of increase in the number of cells, we were justified in expecting a mounting rate of total physiological activity as growth proceeded, until the normal rate of development was checked by the accumulation of unfavorable by-products. The method of renewal of energy-supplying material has been used, for example, with marked success by Bonazzi ('19) in his investigations of the organisms of nitrification.

The second improvement was designed to furnish a more complete picture of growth processes of *Azotobacter* than is obtainable by the determination of one, or even two, metabolic products at the end of an arbitrarily chosen incubation period. Unfortunately, the "micro" determination of carbohydrate and nitrogen on the same sample proved more difficult than was expected, and had to be postponed for the time being. The present work, therefore, lacks this improvement over that reported previously.

The third improvement was to facilitate experimental manipulation and possibly obtain even better results from mechanical agitation. The type of agitation decided upon was a slow

rate of revolution of round-bottomed culture flasks held in a horizontal position. A rotating device was constructed, which held a vertical wooden wheel, to the rim of which were attached, horizontally, four one-liter round-bottomed flasks. The wheel revolving at the rate of one revolution in five minutes kept the culture medium in a slow, uniform motion.

Only one experiment was conducted, and, as pointed out above, it was lacking in the second improvement. Carbohydrate only was determined at short intervals, and the only renewal of this material was in two of the flasks on the rotator. The results obtained were so consistently contrary to expectation that the data from even the one experiment is of some interest; that is, as measured by carbohydrate consumption, the cultures showed a declining instead of an increasing metabolic activity.

The *Azotobacter* culture used was a later generation of the same strain used in previous work. The nutrient solution was prepared as follows:

Solution A:	
Dipotassium phosphate	0.2 gm.
Sodium chloride	0.2 gm.
Dextrose	20.0 gms.
Water, distilled	500.0 cc.
Solution B:	
Magnesium sulphate	0.2 gm.
Calcium sulphate	0.1 gm.
Water	500.0 cc.
Ferric chloride (10 per cent sol.)	3 drops

Solutions *A* and *B* were mixed, and 100-cc. portions then placed in each of four one-liter round-bottomed flasks and in each of two two-liter Erlenmeyers, all flasks receiving in addition 1 gm. calcium carbonate. The flasks were then plugged with cotton and capped with beakers in the usual way. After sterilization by the intermittent method, all except one round-bottomed flask were inoculated with a spiral of a suspension prepared from an agar slant, the round-bottomed flasks placed in position on the rotator, and the Erlenmeyers on a shelf near by. The whole experiment was set up in a warm room kept at 28–30° C. by a thermostat.

The experiment was started March 14, 1919. Two days later a distinct turbidity appeared in the inoculated flasks on the

rotator, and a lesser one in the Erlenmeyers on the shelf. At short intervals 5-cc. samples were removed aseptically and submitted to duplicate sugar determinations by the modified Shaffer method described in the previous paper (Allen, '19). Cultures II and IV received on March 24, 25 cc. of an 8 per cent dextrose solution. Culture No. III was unfortunately lost at this point.

The data are reported as dextrose remaining per 100 cc. of culture solution, no correction being made for evaporation; that is, if a 5-cc. sample is found to contain 35 mgs., the value reported is 0.7000 gm., regardless of the total volume of the culture at the time.

The results appear in tabular form in tables I and II, and in graphical form in fig. 1.

TABLE I

NUMBER GRAMS RESIDUAL DEXTROSE PER 100 CC. CULTURE SOLUTION AT DIFFERENT PERIODS

Culture no.	March 17	March 19	March 24	March 28	April 2
Rotator: (round-bottomed flasks)					
I (Check)	2.1565				
II	1.2732	0.7600	0.110–2.060	1.976	1.255
III	1.4822	1.3507	0.908–Lost		
IV	0.9410	0.5700	0.069–2.023	1.842	1.358
Shelf: (Erlenmeyer flasks)					
V	1.7257		1.636	1.674	1.523
VI	1.5962		1.418	1.507	1.389

TABLE II

MILLIGRAMS DEXTROSE CONSUMED PER DAY IN EACH PERIOD

Culture no.	First 3 days	Next 2 days	Next 5 days	Additional 4 days	Additional 5 days
I Check					
II	295	256	130	21	144
III	225	65	88		
IV	405	185	100	45	97

In constructing the graphs the curves were plotted in the normal manner, with the amounts of dextrose on the Y axis and time on the X axis for the first ten days of the incubation period. In order to make the curves for cultures II and IV continuous and harmonic, the Y ordinate is shifted for these

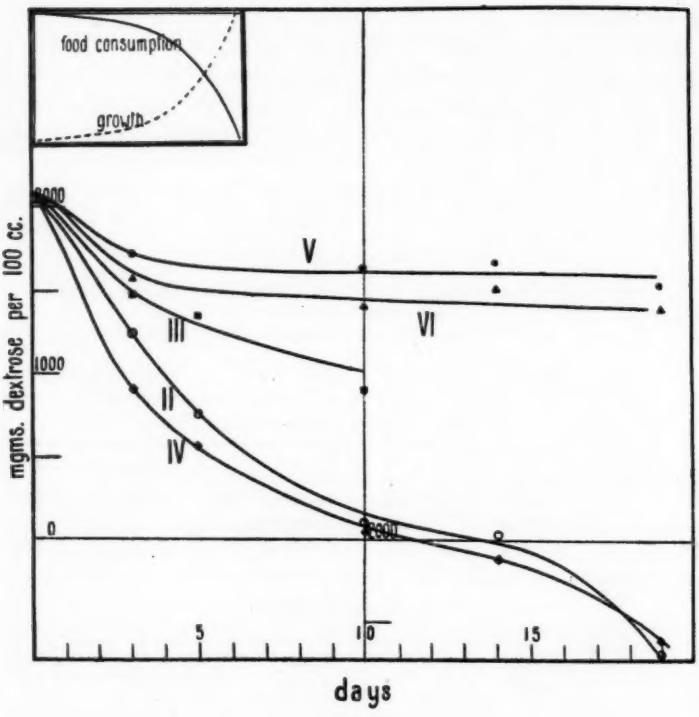

FIG. 1

cultures at the end of 10 days so that the 2000-mgm. point is on the original X axis. The dotted curve in the insert is the Duclaux curve for increase in number of cells, while the solid line represents the type of curve predicted on the assumption that carbohydrate consumption is a simple function of cell multiplication.

The results show that the rate of carbohydrate consumption in *Azotobacter* cultures does not proceed in a manner similar to

the rate of increase in cell numbers predicted by Duclaux for bacterial cultures in general. Indeed, the curves resemble the antipode of the Duclaux curve.

It might be argued that the decrease in rate of sugar consumption with increase of time in the cultures is due to accumulation of by-products, and that this decreased activity corresponds to the falling away from the purely mathematical curve in older cultures, as discussed by Duclaux. The production of toxic substances within seven days in cultures as slow growing and as dilute as these seems to us to be unlikely. Moreover, the fact that the cultures are able to utilize a second portion of sugar seems to us to be opposed to the idea of growth-inhibiting substances in the medium.

The results show also that the cultivation of the organism under the influence of mechanical agitation of a certain type influences to a marked extent the carbon assimilation in its time relations.

On the whole, the experiment emphasizes the need for more studies on the periodic changes in the culture of this organism.

In conclusion, the writer wishes to express his thanks to Professor P. A. Shaffer for rendering this work possible; to Professor B. M. Duggar for suggesting the method of attack, and to Mr. A. Bonazzi for suggestions in regard to the arrangement of the manuscript.

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